8

Organic Acids in Fruits: Metabolism, Functions and Contents

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ABSTRACT

Fleshy fruits and their products are important constituents of the human diet and are also of considerable commercial importance. A large number of different organic acids are present in the fleshy parts of all fruits, but the contents of these can vary greatly both between fruits of different species and their cultivars. The presence of organic acids in the fleshy parts of fruits affects both their palatability and their utilization in fruit products. The organic acids malic, citric, isocitric, galacturonic, quinic, oxalic, and tartaric are very abundant in some fruits, and the phenolic acids and ascorbic acid are of ubiquitous occurrence in fruits. The metabolism, functions and contents of organic acids in the fleshy parts of fruits are outlined in this chapter.

KEYWORDS: ascorbic acid, chlorogenic acid, citric acid, fleshy fruits, galacturonic acid, isocitric acid, malic acid, oxalic acid, organic acids, phenolic acids, quinic acid, tartaric acid

ABBREVIATIONS

CoA	Coenzyme A
3-CQA	3-*O*-caffeoyl-quinic acid
4-CQA	4-*O*-caffeoyl-quinic acid
5-CQA	5-*O*-caffeoyl-quinic acid
DAHP	3-Deoxy-D-arabino-heptulosonic acid 7-phosphate

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DHAR	Dehydroascorbate reductase
DHQ	Dehydroquinic acid
DHS	Dehydroshikimic acid
GDP	Guanosine diphosphate
ICL	Isocitrate lyase
MDH	Malate dehydrogenase
MDHAR	Mondehydroascorbate reductase
MDH	Malate dehydrogenase
ME	Malic enzyme
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
OAA	Oxaloacetate
PEP	Phosphoenolpyruvate
PEPC	Phosphoenolpyruvate carboxylase
PEPCK	Phosphoenolpyruvate carboxykinase
PPDK	Pyruvate, orthophosphate dikinase
UDP	Uridine diphosphate

I. INTRODUCTION

Fleshy fruits and their products are of considerable commercial value and are important constituents of the human diet (Tucker 1993). The contents of organic acids and their derivatives in the flesh of fruits have important dietary considerations, affect the taste of the fruit and in some cases its suitability for processing into various fruit products (Noonan and Savage 1999; Arrigoni and De Tullio 2002; Robbins 2003; Franceschi and Nakata 2005; Sweetman *et al.* 2009; Nguyễn and Savage 2013a).

An organic acid is a molecule that dissociates in aqueous solution to release one or more protons. For many organic acids this results from the dissociation of a carboxylic acid group but for others, such as ascorbic acid, this is not the case (Ulrich 1971; Linster and Van Schaftingen 2007). The degree of this dissociation is largely dependent on both the acid in question and the pH of the solution in which it is dissolved: the lower the pH the less dissociated is the acid. Hence, in the cytoplasm – which has a higher pH – many organic acids are present almost entirely as the anion, whereas in the vacuole – which has a lower pH– a much higher proportion of undissociated acid is present. Organic acids are often referred to by the name of their anions, such as citrate or malate, and this is the form that acts as a substrate for most enzymes and transport proteins (Walker and Chen 2002).

A large number of organic acids are present in the fleshy parts of all fruits, but most of these are in low abundance (Vickery and Pucher 1940; Thimann and Bonner 1950; Nitsch 1953; Ulrich 1971). In this chapter, attention is focused on those organic acids that can be abundant. The different organic acids present in fruits function in numerous and often unrelated metabolic processes, and include compounds that act as intermediates in diverse metabolic pathways, precursors for the synthesis of amino acids, many plant hormones (e.g., auxins, gibberellins and

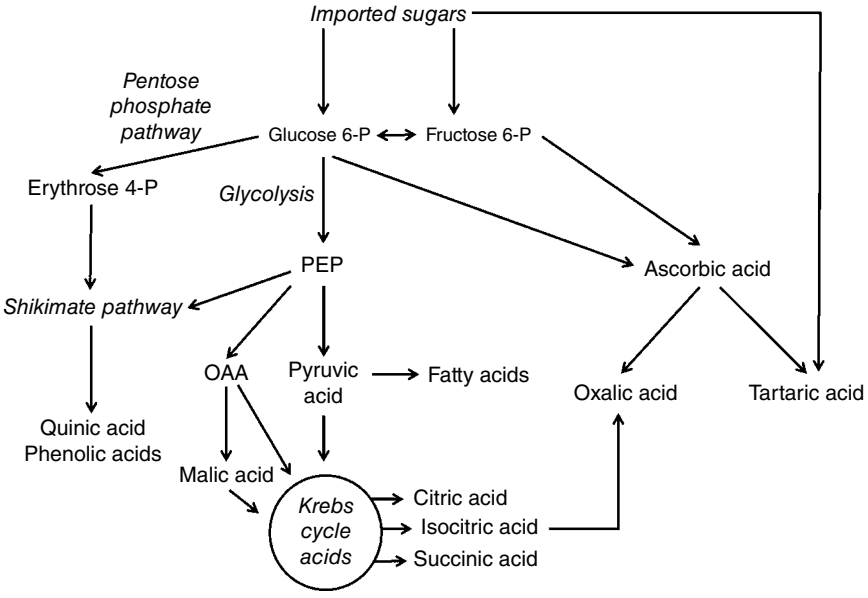

Fig. 8.1. Simplified scheme showing the pathways utilized in the synthesis of the main groups of organic acids found in fruits. OAA = Oxaloacetate; PEP = Phosphoenolpyruvate.

salicylic acid), fatty acids, a large number of secondary metabolites, and certain cell wall components (Ulrich 1971). These acids can be divided into groups based on the pathways utilized in their synthesis, and a simplified scheme illustrating this is shown in Fig. 8.1.

The total amount of organic acids that are present in the fleshy parts of fruits of different species and their cultivars can vary greatly, as can the relative abundance of the individual organic acids. In addition, these contents are dependent on both the tissue of the fruit and its stage of development, as well as diverse environmental factors (Sweetman *et al.* 2009; Ford 2012; Etienne *et al.* 2013; Famiani *et al.* 2015). Malic and/or citric acid are abundant in the flesh of most fruits (Vickery and Pucher 1940; Thimann and Bonner 1950; Sweetman *et al.* 2009; Etienne *et al.* 2013; Famiani *et al.* 2015). For example, their content can account for up to 40–50% of the dry weight of the unripe flesh of fruits such as apricot (Baldicchi *et al.* 2015). In many fruits quinic acid can be abundant, and can account for 20–30% of the dry weight of the flesh of some citrus fruits early in development (Albertini *et al.* 2006). In a smaller number of fruits, either oxalic or tartaric acid can be very abundant (Thimann and Bonner 1950; Ulrich 1971; Nguyễn and Savage 2013a; Van den Bilcke *et al.* 2014).

The aim of this chapter is to provide a concise account of the metabolism, functions, and contents of malic, citric, quinic, phenolic, ascorbic, oxalic, and tartaric acids in the fleshy parts of fruits.

II. THE FUNCTION OF THE FLESH OF FRUITS AND ITS IMPLICATION FOR THEIR ORGANIC ACID CONTENTS

The fruits of many plants serve both to protect and assist in the dispersal of the seeds. Certain fruits are fleshy (e.g., plum, grape, and blueberry), whilst others are not (e.g., pea, maize, and *Arabidopsis*) (Vines and Rees 1968; Coombe 1976). In this chapter, fleshy fruits refer both to fruits that are used as either a dessert or a vegetable, and to fruits of both cultivated and wild plants. Non-fleshy fruits can assist in the dispersal of the seeds in diverse ways. For example, some fruits possess fibrous papery outgrowths of the ovary wall (e.g., sycamore), and these assist in the dispersal of the seed by wind. The functions of the fleshy parts of fruits are to attract animals that disperse their seeds and also to protect the seeds (Coombe 1976; Cipollini and Levey 1997; Barry 2010; Bolmgren and Eriksson 2010). Fleshy fruits have evolved independently several times (Mack 2000), and in the case of a large number of commercially grown fruits their characteristics have been extensively modified by human selection and breeding over many thousands of years (Bollard 1971; Janick 2005; This *et al.* 2006; Wu *et al.* 2014). The fleshy parts of fruits can be derived from various tissues, and which tissue gives rise to the flesh depends on the fruit in question. In berry fruits (e.g., grape, currant, kiwifruit, tomato, and blueberry) and drupes (e.g., plum, cherry, peach, raspberry, and blackberry), the fleshy part of the fruit is derived from the ovary wall (pericarp). A drupe is a berry in which the inner part of the ovary wall (endocarp) becomes woody. In some other fruits (e.g., pome fruits: apple, pear, and medlar) the fleshy tissue is derived from the receptacle (Vines and Rees 1968; Bollard 1971; Coombe 1976). The fleshy parts of fruits ripen when the seeds are approaching maturity. Thus, the flesh of unripe fruits is less attractive to animals and hence contributes to protecting the developing seed. Various features of unripe flesh, such as a hard texture, sour taste and green color can contribute to making it less attractive. A number of changes occur to the flesh during ripening that make it attractive to animals. These changes often include a softening of the flesh, the development of an attractive coloration, the release of volatile compounds with an attractive odor, and a change to a sweeter taste (Vines and Rees 1968; Coombe 1976; Willson and Whelan 1990; Tucker 1993; Smith

et al. 2003; Barry 2010). The flesh of diverse fruits show these changes during ripening. Many of these fruits have evolved independently of each other (Mack 2000), and this indicates that this group of characteristics has also evolved on different occasions and that these characteristics are of widespread importance in attracting animals.

The organic acid content of fruits contributes to both their taste and flavor (Bollard 1971; Ford 2012; Mikulic-Petkovsek *et al.* 2012; Famiani *et al.* 2015). The sourness of a fruit depends largely on the relative amounts of organic acids and sugars present (Mikulic-Petkovsek *et al.* 2012; Famiani *et al.* 2015). Thus, if a sour fruit is harvested it can be made to taste sweet if sugar (usually sucrose) is added to it. The flesh of many fruits selected for human consumption accumulates large amounts of imported sugars. Further, starch is converted to soluble sugars in the flesh of fruits in which it is accumulated before ripening; examples are tomatoes, apples, kiwifruits and bananas, although in the latter fruit there is only a partial conversion (Hubbard *et al.* 1990; Antognozzi *et al.* 1996; Berüter and Feusi 1997; Schaffer and Petreikov 1997). In the flesh of many fruits the amounts of abundant acids per gram fresh weight (FW) such as malic and citric decrease during ripening. This decrease can arise either as a result of metabolism of the acid, or from a dilution effect arising from the expansion of the flesh during fruit development and ripening (Famiani *et al.* 2005, 2015; Sweetman *et al.* 2009). However, in some fruits there is little decrease in organic content per gram FW (i.e., in concentration), and in some it actually increases (Famiani *et al.* 2015). Nevertheless, in many of these fruits the increase in soluble sugar concentration during ripening complements the presence of malic and citric acids and imparts on the flesh a sweet taste. Thus, in the unripe flesh of many fruits a higher content of certain organic acids and a low content of sugars imparts on the flesh a sour flavor, and this contributes to making them less palatable to animals.

Present-day citrus fruits have arisen as a result of selection and breeding by humans over many thousands of years (Wu *et al.* 2014). Certain sweet cultivars of orange, lemon and lime contain very low amounts of both malic and especially citric acids in their flesh, whereas other cultivars can have high contents (Albertini *et al.* 2006). Thus, the very different contents of malic and citric acid in present-day citrus fruit cultivars likely arose as a result of selection and breeding by humans. The very low amounts of malic and citric acid in the flesh of some of these fruits indicates that, in the flesh of others in which these contents are high, the bulk of these contents is not necessary for their development and metabolism. However, a certain proportion of the content of

these acids must have underlying metabolic functions. In the case of other acids such as quinic, tartaric, oxalic and the phenolic acids and their derivatives it is possible that, in certain fruits, a proportion of their content is associated with altering the palatability of the fruit and with other interactions with animals and microbes. Such interactions include deterring insect and microbial attack and imparting an attractive color on the fruit, often through further metabolism of the acid to a colored compound (e.g., anthocyanins) (Guo *et al.* 2014).

Fleshy fruits contain tissues other than their flesh, and there must be a tissue that prevents the seeds from being destroyed by the animal that disperses it (Vines and Rees 1968). Seeds that are ingested by the animal have a woody coating, the origin of which is often either the seed coat itself (e.g., berries such as grapes, tomatoes and currants) or the inner part of the ovary wall (e.g., drupes such as blackberries and raspberries). In other cases (e.g., the drupes cherry, plum, and peach) the seed is usually not ingested by the animal but is discarded along with the woody endocarp that encloses and protects it (Vines and Rees 1968). The organic acid contents of these seeds and woody endocarps are very different from the fleshy parts (Noona and Savage, 1999; Walker *et al.* 2011a,b; Famiani *et al.* 2012).

III. ACIDS THAT CONTAIN A BENZENE RING: THE AROMATIC ACIDS

A number of organic acids present in the flesh of fruits contain a benzene ring, derived from intermediates of the shikimate pathway. This pathway is located in the plastid and consists of seven enzymatic reactions that convert phosphoenolpyruvate (PEP; produced by glycolysis) and erythrose 4-phosphate (derived from the pentose phosphate pathway) to chorismate (Fig. 8.2) (Widhalm and Dudareva 2015). The shikimate pathway produces metabolites that are used in the synthesis of aromatic amino acids, anthocyanins, lignin and numerous other metabolites.

A. Quinic and Shikimic Acids

1. Occurrence and Functions of Quinic Acid in Plants. Quinic acid is accumulated in a number of organs such as the leaves, fruits and tubers, and its concentration differs greatly between plant species (Yoshida *et al.* 1975; Farré *et al.* 2001; Marsh *et al.* 2009). In those tissues in which its subcellular location has been determined, the bulk of the

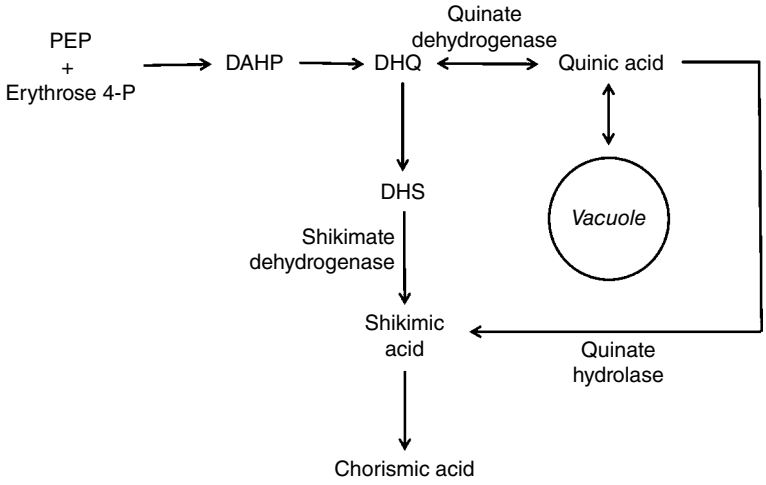

Fig. 8.2. Simplified scheme showing the shikimate pathway and the synthesis of quinic and shikimic acids. PEP = Phosphoenolpyruvate; DAHP = 3-Deoxy-D-arabino-heptulosonic acid 7-phosphate; DHS = Dehydroshikimic acid; DHQ = Dehydroquinic acid.

quinic acid is located in the vacuole (Moskowitz and Hrzadina 1981; Yamaki 1984; Farré *et al.* 2001; Fortes *et al.* 2011). Quinic acid is thought to act as a reserve compound for phenolic biosynthesis in some plant tissues, including fruits (Boudet *et al.* 1985; Ossipov *et al.* 1995; Marsh *et al.* 2009; Boudet 2012; Zulet *et al.* 2013). For example, in the developing needles of a number of conifers, large amounts of quinic acid that accumulate in the vacuole (10–14% of dry mass) are utilized as the needle matures (Ossipov *et al.* 1995). In fruits of different kiwifruit species, the quinic acid content per fruit decreases during development and could function as a storage compound that is subsequently utilized in the synthesis of phenolics (Marsh *et al.* 2009). Nevertheless, even in fruits in which there is no decrease in content per fruit (when this decrease is determined as the difference in content measured at two points several days apart) quinic acid could also serve the same purpose. This is because there could be times when there are short-term effluxes of quinic acid from the vacuole and it is utilized, and periods when quinic acid is synthesized and transferred to the vacuole.

2. Synthesis and Catabolism of Quinic Acid. Quinic acid is synthesized by a reaction that branches off the shikimate pathway (Fig. 8.2). Quinate dehydrogenase converts dehydroquinic acid (an intermediate of the shikimate pathway) to quinic acid, which can be stored in the

vacuole (Ossipov *et al.* 1995; Marsh *et al.* 2009; Boudet 2012). Stored quinic acid can re-enter the shikimate pathway by the action of either quinate deydrogenase or, in some plant tissues, quinic hydrolase (Leuschner *et al.* 1995; Boudet 2012; Guo *et al.* 2014). A different fate for quinic acid is its direct use in the synthesis of compounds such as the chlorogenic acids (conjugates of quinic acid and cinnamic acids) (Guo *et al.* 2014).

3. Occurrence and Contents of Quinic and Shikimic Acids in Fruits. Quinic acid is present in the flesh of most fruits and, in the flesh of some it is abundant (Okuse and Ryugo 1981; Moing *et al.* 1999; Albertini *et al.* 2006; Garcia-Marino *et al.* 2008; Chen *et al.* 2009). Typical concentrations of quinic and shikimic acids in the flesh of fruits are shown in Tables 8.1 and 8.2, and from these it is evident that in most fruits shikimic acid is usually much less abundant than quinic acid. For those fruits in which it has been studied it is clear that the content of quinic acid is often dependent on the genotype/cultivar of a given type of fruit. Further, this content varies between tissues of a fruit, and is also dependent on their stage of development. In addition, quinic acid content can also be affected both by the conditions under which the fruit is grown and by storage and processing of the fruit after harvest. For several fruits some of these factors are considered below.

Table 8.1. Concentrations of quinic acid (QA; mg g^{-1} FW) in the flesh of some ripe fruits.

Fruits	QA level	Reference
Apple	0.17–0.66	Fuleki *et al.* 1994
Babaco	0–1.5	Rodriguez *et al.* 1992
Blackberry	0.01–0.2	Wrolstad *et al.* 1980
Blueberry (highbush)	0.26–0.39	Markakis *et al.* 1963
Blueberry (lowbush)	ca. 10	Kalt and McDonald 1996
Citrus (various)	2.0–7.0	Albertini *et al.* 2006
Cranberry (European)	3.8–13.3	Česonienė *et al.* 2015
Currant (black)	0–1.5	Rodriguez *et al.* 1992
Kiwifruit	4.0–13.0	Marsh *et al.* 2009
Medlar	1.2	Rodriguez *et al.* 1992
Peach	3.0	Byrne *et al.* 1991
Peach	1.0–2.0	Dirlewanger *et al.* 1999
Pear	0.38–0.48	Drake and Eisele 1999
Plum (damson)	5.0	García-Mariño *et al.* 2008
Plum (Japanese)	1.6–5.2	Robertson *et al.* 1992
Quince	Trace–0.6	Silva *et al.* 2002
Strawberry	0.25	Moing *et al.* 2001
Tamarillo	3.7–8.2	Rodriguez *et al.* 1992
Whortleberry	5.2–11.3	Rodriguez *et al.* 1992

Table 8.2. Concentrations of shikimic acid (SA; mg g^{-1} FW) in the flesh of some ripe fruits.

Fruits	SA level	Reference
Apple	0.003–0.02	Fuleki *et al.* 1995
Blackberry	0.028–0.089	Mikulic-Petkovsek *et al.* 2012
Blueberry (lowbush)	0.07	Kalt and McDonald 1996
Currant (red)	0.353	Mikulic-Petkovsek *et al.* 2012
Currant (black)	0.027	Mikulic-Petkovsek *et al.* 2012
Currant (white)	0.281	Mikulic-Petkovsek *et al.* 2012
Cherry (sweet)	0.5–2.0	Kelebek and Selli 2011
Cherry (sweet)	0.015–0.048	Ballistreri *et al.* 2013
Gooseberry	0.580–0.722	Mikulic-Petkovsek *et al.* 2012
Jostaberry	0.844	Mikulic-Petkovsek *et al.* 2012
Pear	0.04–0.07	Drake and Eisele 1999
Quince	0.01–0.02	Silva *et al.* 2002
Raspberry	0.014–0.020	Mikulic-Petkovsek *et al.* 2012
Strawberry	0.004–0.018	Sturm *et al.* 2003

Stone fruits. Plums and peaches can contain appreciable amounts of quinic acid (De Moura and Dostal 1965; Moing *et al.* 1998; Garcia Mariňo *et al.* 2008; Singh *et al.* 2009). In the ripening flesh of 12 peach cultivars the concentration of quinic acid was between 2.2 and 5.3 mg g^{-1} FW (Byrne *et al.* 1991). The quinic acid concentration of whole ripe fruits of a range of Japanese plum genotypes contained 1.6–5.2 mg g^{-1} FW (Robertson *et al.* 1992). The quinic acid concentration (g^{-1} FW) at several stages of the development of the flesh + skin of four stone fruits (apricot, plumcot [Japanese plum/apricot hybrid], Japanese plum and peach) has been reported (Bae *et al.* 2014). Quinic acid was several-fold more abundant in peach and plum than in apricot. In all these fruits it appears that the quinic content of the entire flesh + skin does not decrease during most of development (Bae *et al.* 2014), and therefore most of the decrease in concentration arises from a dilution effect brought about by the expansion of the flesh. In peach ('Monroe') flesh, the quinic acid concentration decreased from 10 mg g^{-1} FW at 80 days after flowering to 2 mg g^{-1} FW 60 days later in ripe fruit at 140 days (Chapman and Horvat 1990). However, there was no decrease in the quinic content per whole flesh, and the decrease could be accounted for by the expansion of the flesh (Chapman and Horvat 1990).

Pome fruits. Apples contain appreciable amounts of quinic acid (Hulme and Wooltorton 1957; Wu *et al.* 2007). In five cultivars of apple, quinic acid (g^{-1} FW) decreased during ripening, and it was suggested that it was used in the synthesis of chlorogenic acid (Blanco *et al.* 1992).

In general, pears contain appreciable amounts of quinic acid, but its abundance can differ greatly among cultivars (Arfaioli and Bosetto 1993; Chen *et al.* 2007; Sha *et al.* 2011a). Drake and Eisele (1999) reported values of 0.38–0.48 mg g^{-1} FW in ripe pear flesh. Quinic acid is also present in appreciable amounts in medlar (Barbieri *et al.* 2011).

Soft fruits. In the ripe berries of 40 genotypes of European cranberry (*Vaccinium oxycoccos*) the quinic acid concentration was between 3.8 and 13.3 mg g^{-1} FW (Česonienė *et al.* 2015). Forney *et al.* (2012) found the quinic acid concentration to be about 4 mg g^{-1} FW in 'Stevens' cranberry (*Vaccinium macrocarpon*), and that its content did not decrease during ripening. In highbush blueberry (*Vaccinium corymbosum*) the quinic acid concentration decreased from about 1.70 mg g^{-1} FW in white fruit to about 0.2 mg g^{-1} FW in mature fruit (Forney *et al.* 2012).

Kiwifruit. Quinic acid is abundant in kiwifruit species (*Actinidia* spp.) (Reid *et al.* 1982; Marsh *et al.* 2009; McGhie 2013). In *A. chinensis* its concentration ranged during development between 4 and 7 mg g^{-1} FW. Further, the content per whole fruit decreased as it matured, which suggests that it is metabolized (Reid *et al.* 1982). Quinic acid is abundant in fruits of *A. chinensis*, *A. deliciosa* and *A. arguta* × *A. melandra*, and in mature fruits of these its concentration is 7–14 mg g^{-1} FW (Marsh *et al.* 2009). In *A. chinensis* the content of quinic acid decreased by about half as the fruit matured, and in the other kiwifruit species any decrease was much less (Marsh *et al.* 2009). In general, in fruits at the eating-ripe-stage, compared to *A. deliciosa* and *A. chinensis*, the proportion of quinic acid is higher in *A. rufa* and lower in *A. arguta* (Nishiyama *et al.* 2008).

Citrus fruits. In the flesh of lemon, orange and lime, quinic acid is the most abundant acid during the first 50 days of development (orange 17–27 mg g^{-1} FW; lemon 18–27 mg g^{-1} FW; lime about 12 mg g^{-1} FW), but its concentration (g^{-1} FW) decreases markedly as the fruit expands and develops (Albertini *et al.* 2006). A large part of this decrease is brought about by the expansion of the flesh. The quinic acid concentration of mature Ponkan mandarin (*Citrus reticulata*) flesh is around 3 mg g^{-1} FW (Chen *et al.* 2012).

Other fruits. Quinic acid is present in grapes but is at low abundance (Ruffner 1982a). In saskatoon (*Amelanchier alnifolia*), quinate concentration declined from about 1.5 mg g^{-1} FW in immature fruits to undetectable amounts as the fruit matured (Rogiers and Knowles 1997).

The quinic acid concentration in pineapple flesh is 0.52–1.40 mg g^{-1} FW (Sun *et al.* 2016). The average quinic acid concentration in the ripe flesh of 18 loquat cultivars was 2.04 mg g^{-1} FW (Li *et al.* 2015).

B. The Phenolic Acids

The phenolic acids, together with other phenolics, are associated with the color, taste and nutritional properties of fruits, and can also contribute to their protection from animals and microbes (Robbins 2003). The phenolic acids are one subclass of the phenolics. Another subclass whose members are abundant in fruits are the flavonoids, which include the anthocyanins (Gross 1981; Häkkinen *et al.* 1999; Robbins 2003; Rothwell *et al.* 2013). The defining feature of a phenolic is the possession of a phenol (an aromatic ring containing at least one hydroxyl), and the phenolic acids also possess a carboxylic acid functionality (Gross 1981; Robbins 2003). The majority of phenolic acids are based on either of two carbon frameworks: the hydroxycinnamic and hydroxybenzoic structures (Gross 1981; Robbins 2003). Those phenolic acids based on the hydroxycinnamic structure include cinnamic, coumaric, ferulic and caffeic acids, whilst those based on the hydroxybenzoic acid structure include benzoic, salicylic, syringic, and gallic acids (Robbins 2003; Rothwell *et al.* 2013). Often, only a small fraction of these compounds exist as the free acid, and the majority are chemically linked to molecules such as glucose, tartaric acid, other phenolics, and terpenes. Chlorogenic acid is the name given to a large group of phenolic acids which are formed by the conjugation of certain cinnamic acids and quinic acid (Clifford 2000). A common form of these compounds is 5-*O*-caffeoyl-quinic acid (5-CQA: a conjugate of caffeic acid and quinic acid), and this is often referred to as chlorogenic acid (Clifford 2000). Other related compounds are 3-*O*-caffeoyl-quinic acid (3-CQA) and 4-*O*-caffeoyl-quinic acid (4-CQA), and the relative abundance of these and 5-CQA differs widely among fruits of different species of plant (Macheix *et al.* 1990). Several subgroups of the cholorogenic acids can be recognized, and these include conjugates of quinic acid (or its close relatives) with one or more of these cinnamic acids, such as ferulic and coumaric acids. Often, more than one quinic acid can be conjugated to one of these cinnamic acids (Clifford 2000; Plazas *et al.* 2013).

1. Functions of Phenolic Acids in Fruits and Other Plant Tissues. The phenolic acids have numerous and diverse functions in plants (Gross 1985; Macheix *et al.* 1990; Robbins 2003; Widhalm and Dudareva 2015).

An important function is as precursors for the synthesis of many other phenolic compounds such as anthocyanins, lignin, ubiquinone (respiratory cofactor) and folic acid (vitamin B_9) (Gross 1985; Robbins 2003; Widhalm and Dudareva 2015). The acids themselves can function in free radical scavenging, the inhibition of lipid peroxidation, as components of the cell wall, in the enzymatic browning of both fruits and vegetables, and in plant resistance to animals and microbes (Macheix *et al.* 1990; Bushman *et al.* 2002; Plazas *et al.* 2013; Widhalm and Dudareva 2015). Ferulic acid is an important component of many plant cell walls (Mathew and Abraham 2004). The hydroxybenzoic acids include the hormone salicylic acid (Widhalm and Dudareva 2015).

2. Synthesis of Phenolic Acids. The building blocks of phenolic acids (e.g., cinnamic, gallic and benzoic acids) are derived from intermediates of the shikimate pathway (Fig. 8.3) (Gross 1981; Tohge *et al.* 2013). However, many phenolic acids are conjugated with compounds such as sugars and tartaric acid that are synthesized by different pathways. In addition, different pathways can be utilized to produce both some of the individual phenolic acids themselves and their conjugated forms (e.g., 5-CQA), and where studied it appears that which pathway predominates depends on both the plant species and tissue (Mahesh *et al.* 2007; Sonnante *et al.* 2010; Widhalm and Dudareva 2015). For example, there are four related pathways by which 5-CQA can be synthesized in plants (Fig. 8.4) (Mahesh *et al.* 2007).

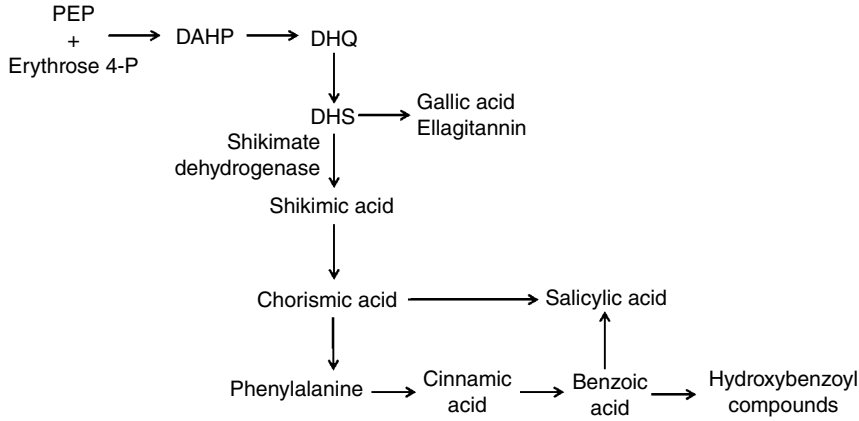

Fig. 8.3. Simplified scheme showing the synthesis of phenolic acids. PEP = Phosphoenolpyruvate; DAHP = 3-Deoxy-D-arabino-heptulosonic acid 7-phosphate; DHS = Dehydroshikimic acid; DHQ = Dehydroquinic acid.

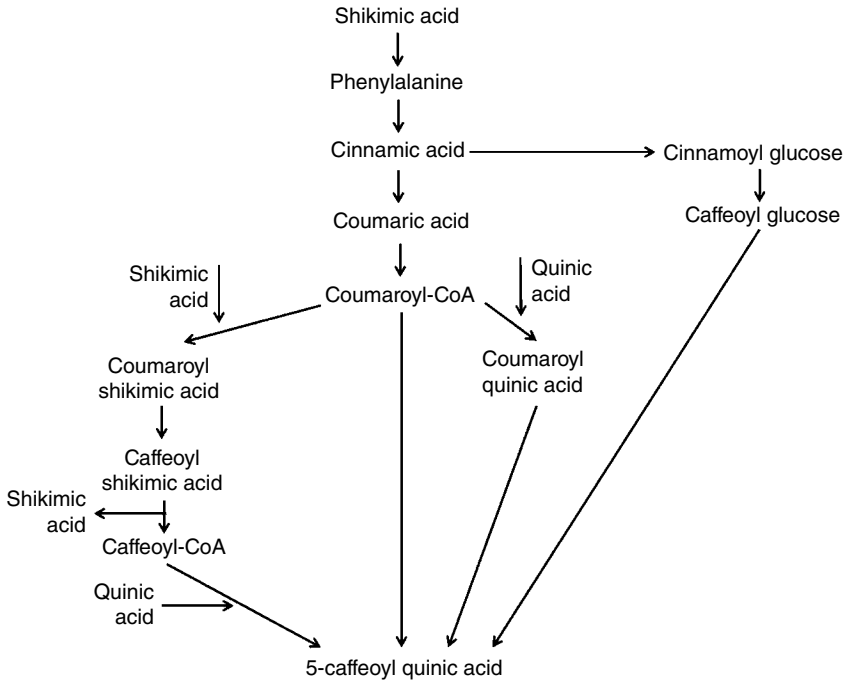

Fig. 8.4. Simplified scheme showing the synthesis of 5-caffeoyl quinic acid. CoA = Coenzyme A.

3. Occurrence of Phenolic Acids in Fruits. The total phenolic content of whole fruits of different species varies considerably. For example, in the berries of 27 genotypes of plants the concentration ranged from 0.4 to 13 mg gallic acid equivalents g^{-1} FW (Mikulic-Petkovsek *et al.* 2012, 2015). In addition, the phenolic contents of different parts of fruits (e.g., flesh, skin or seeds) are not the same, and these contents also change during development (Abe *et al.* 2012). The total phenolic content of the flesh differs between fruits of different plant species and cultivars of a given fruit (van Buren 1971; Gil *et al.* 2002; Vieira *et al.* 2009). For example, the concentration of total phenolics in the flesh of different cherry cultivars was 0.65–3.01 mg gallic acid equivalents g^{-1} FW (Chaovanalikit and Wrolstad 2004). In some fruits the bulk of the phenolic content consists of phenolic acids and flavonoids, and in these the proportion of flavonoids to phenolics can vary considerably among fruits of different species (Häkkinen *et al.* 1999). For example, de-seeded berries of sea buckthorn and cranberry contain mainly flavonoids, whereas those of raspberry and strawberry contain mainly phenolic acids (Häkkinen *et al.* 1999). Further, the contents of individual phenolic acids are not the same in fruits of different species

(Häkkinen *et al.* 1999; Rothwell *et al.* 2013). In some fruits, including pomegranate, strawberry, blackberry, blackcurrant, raspberry and fruits of a number of species of the myrtle family (e.g., guava and jabuticaba), ellagic acid and its derivatives are quite abundant (Häkkinen *et al.* 1999; de Ancos *et al.* 2000; Landete 2011; Abe *et al.* 2012; Lee *et al.* 2012; Mikulic-Petkovsek *et al.* 2015). A common derivative of ellagic acid is ellagitannin, and this can be located in the cell wall (Grundhöfer *et al.* 2001). In the edible parts of fruits of a range of species of the myrtle family the concentration of free ellagic acid and its derivatives was 0.22–3.11 mg g^{-1} FW (Abe *et al.* 2012). Ferulic acid can comprise a large proportion of the phenolic content of the de-seeded parts of some fruits such as blueberry, rowanberry, and chokeberry (Häkkinen *et al.* 1999). The chlorogenic acids and other cinnamic acid conjugates are of widespread occurrence in fruits (Clifford 2000). In various plums, 3-CQA is more abundant than 5-CQA, and in prune the 3-CQA concentration of the edible parts was 1.2–1.5 mg g^{-1} FW (Nakatani *et al.* 2000). In the flesh plus skin of several peach cultivars, 5-CQA concentration decreased from 1.2–5.5 to 0.8–1.8 mg g^{-1} FW as the fruit matured (Villarino *et al.* 2011). 5-CQA is present in many fruits, and its concentration has been reported to be: apple (40–120 mg 100 g^{-1} DW), apricot (2–51 mg 100 g^{-1} DW), cherry (2–9 mg 100 g^{-1} DW), peach (10–160 mg 100 g^{-1} DW), plum (40 mg 100 g^{-1} DW), tomato (20–40 mg 100 g^{-1} DW), and pepper (70–90 mg 100 g^{-1} DW) (Plazas *et al.* 2013). In mature aubergine fruits, 5-CQA accounts for 30–75% of its total phenolic content, and its concentration is 140–2800 mg 100 g^{-1} DW (Plazas *et al.* 2013). Genotype, stage of development and environmental factors can alter the abundance of 5-CQA in aubergine fruits, and this abundance can depend on the tissue of the fruit (Plazas *et al.* 2013).

C. Other Aromatic Acids

Among other groups of aromatic acids can be included phenylacetic acid, an auxin which is synthesized from phenylalanine, and the auxin indole-3-acetic acid which is synthesized from the aromatic amino acid tryptophan (Cook *et al.* 2016).

IV. THE INTER-RELATED ACIDS: ASCORBIC, OXALIC, TARTARIC, AND GALACTURONIC

These acids are often synthesized from glucose and/or fructose by inter-related pathways (Fig. 8.5). However, oxalic acid can also be synthesized from isocitric acid.

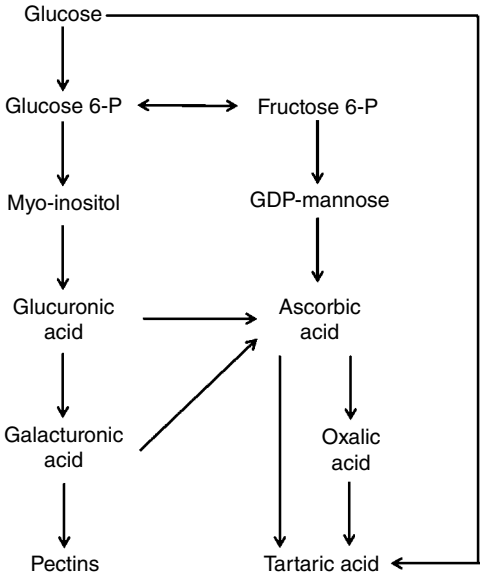

Fig. 8.5. Simplified scheme showing the interrelations between ascorbic, oxalic, tartaric, and galacturonic acids. GDP = Guanosine diphosphate.

A. Ascorbic Acid (Vitamin C)

1. Functions of Ascorbic Acid in Fruits and Other Plant Tissues. Vitamin C is a generic term for all compounds exhibiting the biological activity of ascorbic acid, and dehydroascorbic acid (DHA) exhibits this activity because it is readily converted into ascorbic acid (Fig. 8.6; after Lee and Kader 2000). Ascorbic acid is present in all plant tissues, and is located in all compartments of the cell (Arrigoni and De Tullio 2002). This acid has diverse functions and is an antioxidant in both plants and animals: that is, it functions in preventing damage from free radicals and other reactive oxygen species (Arrigoni and De Tullio 2002; Smirnoff 2011; Ford 2012). Further, ascorbic acid is an important cofactor for a number of enzymes, and ascorbic acid-dependent metabolic reactions influence many processes such as cell division, gene expression, and defense reactions (Arrigoni and De Tullio 2002; Smirnoff 2011).

2. Synthesis of Ascorbic Acid in Fruits and Other Plant Tissues. In plants, four pathways can potentially be used to synthesize ascorbic acid (although it must be noted that the existence of some of these pathways is not fully proven), and the relative importance of each of these

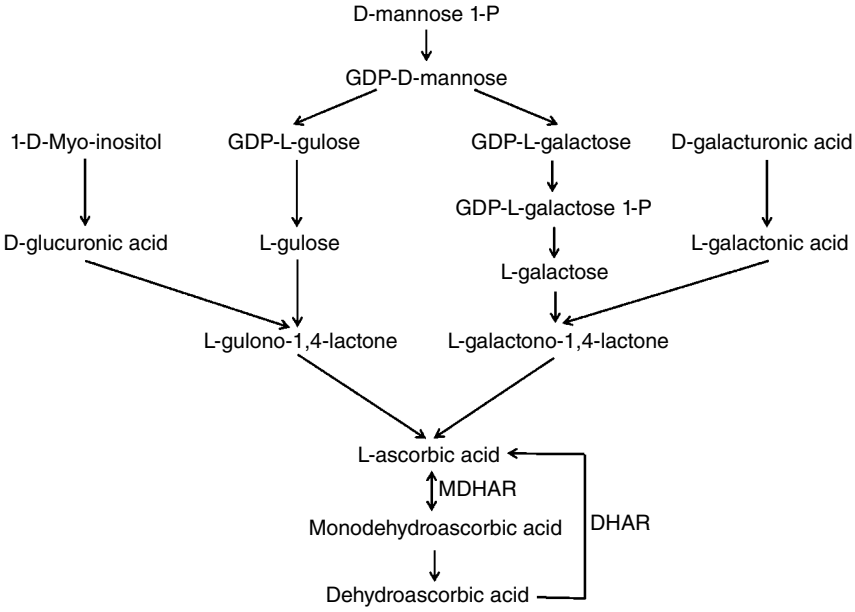

Fig. 8.6. Simplified scheme showing the synthesis and catabolism of ascorbic acid. GDP = Guanosine diphosphate; DHAR = Dehydroascorbate reductase; MDHAR = Mondehydroascorbate reductase.

pathways is dependent on the plant species, tissue, and stage of development (Smirnoff 2011; Alós *et al.* 2014). These pathways are outlined in Fig. 8.6. Ascorbic acid can be transported between plant organs (Arrigoni and De Tullio 2002). However, in blackcurrant fruits and likely also in kiwifruit, the bulk of their ascorbic acid content is thought to be synthesized within the fruit (Hancock *et al.* 2007; Li *et al.* 2010). In orange flesh, immature peach fruits and the fruits of apple, tomato and kiwifruit, the L-galactose pathway can be predominant, though other pathways could be involved (Bulley *et al.* 2009; Imai *et al.* 2009; Ioannidi *et al.* 2009; Badejo *et al.* 2012; Mellidou *et al.* 2012; Alós *et al.* 2014). In strawberry fruits, both the D-galctouronic acid and myo-inositol pathways may be prevalent, but the galactose pathway must also be important (Cruz-Rus *et al.* 2011; Bulley *et al.* 2012). In orange, predominance of the pathways utilized in ascorbic acid synthesis appears to be dependent on both the tissue and stage of development. In the colored part of the peel, early in development, both the myo-inositol and L-galactose pathways appear to be utilized but as the fruit matures the latter predominates (Alós *et al.* 2014). In both tomato and grape, the

L-galactose pathway appears to be predominant in immature fruits, whereas the D-galacturonic acid pathway predominates in ripe fruits (Melino *et al.* 2009; Cruz-Rus *et al.* 2010; Badejo *et al.* 2012). In plant tissues the content of ascorbic acid is affected by its degradation or recycling, in addition to its rate of synthesis (Haroldsen *et al.* 2011; Smirnoff 2011; Yang *et al.* 2011; Zhang *et al.* 2011; Alós *et al.* 2014).

3. Occurrence and Contents of Ascorbic Acid in Fruits. Some typical values for the ascorbic and DHA concentrations in the ripe flesh of different fruits are shown in Tables 8.3 and 8.4. Nevertheless, these concentrations can be affected by a number of factors. The ascorbic acid and

Table 8.3. Concentrations of ascorbic acid (AA; mg g^{-1} FW) in the flesh of some ripe fruits and in some leaves.

Fruits	AA level	Reference
Banana	0.15	Vanderslice *et al.* 1990
Babaco	0.21–0.32	Rodriguez *et al.* 1992
Blackberry	0.06	Rodriguez *et al.* 1992
Blackberry	0.18	Agar *et al.* 1997
Blueberry (various spp.)	0.017–0.097	Prior *et al.* 1998
Currant (black)	1.5–2.2	Rodriguez *et al.* 1992
Currant (black)	0.86	Agar *et al.* 1997
Currant (red)	0.6–0.8	Rodriguez *et al.* 1992
Currant (red)	0.2–0.5	Nour *et al.* 2011
Currant (white)	0.5	Rodriguez *et al.* 1992
Cherry (sweet)	0.08–0.17	Girard and Kopp 1998
Feijoa	0.16	Rodriguez *et al.* 1992
Grapefruit	0.21	Vanderslice *et al.* 1990
Kiwifruit	0.60	Agar *et al.* 1997
Lemon	0.50	Mitchell *et al.* 1992
Mandarin	0.34	Mitchell *et al.* 1992
Medlar	0.003	Rodriguez *et al.* 1992
Melon (Cantaloupe)	0.31	Vanderslice *et al.* 1990
Orange	0.55–0.75	Vanderslice *et al.* 1990
Pepper	1.29–1.51	Vanderslice *et al.* 1990
Persimmon	1.1	Wright and Kader 1997
Raspberry	0.23–0.35	Rodriguez *et al.* 1992
Strawberry	0.60	Agar *et al.* 1997
Tamarillo	0.02–0.22	Rodriguez *et al.* 1992
Tomato	0.11	Vanderslice *et al.* 1990
Whortleberry	0.009–0.01	Rodriguez *et al.* 1992
Leaves		
Spinach (fresh)	0.62	Gil *et al.* 1999
Spinach (boiled)	0.12	Gil *et al.* 1999
Cabbage (fresh)	0.42	Vanderslice *et al.* 1990
Cabbage (boiled)	0.24	Vanderslice *et al.* 1990

Table 8.4. Concentrations of dehydroascorbic acid (DHA; mg g^{-1} FW) in the flesh of some ripe fruits and some leaves.

Fruits	DHA level	Reference
Banana	0.033	Vanderslice *et al.* 1990
Blackberry	0.030	Agar *et al.* 1997
Currant (black)	0.060	Agar *et al.* 1997
Grapefruit	0.023	Vanderslice *et al.* 1990
Kiwifruit	0.053	Agar *et al.* 1997
Lemon	0.239	Mitchell *et al.* 1992
Mandarin	0.037	Mitchell *et al.* 1992
Melon (Cantaloupe)	0.030	Vanderslice *et al.* 1990
Orange	0.083	Vanderslice *et al.* 1990
Pepper	0.030–0.050	Vanderslice *et al.* 1990
Persimmon	0.110	Wright and Kader 1997
Strawberry	0.050	Agar *et al.* 1997
Tomato	0.030	Vanderslice *et al.* 1990
Leaves		
Spinach (fresh)	0.130	Gil *et al.* 1999
Spinach (boiled)	0.180	Gil *et al.* 1999

DHA content of citrus fruits is dependent on the tissue of the fruit, and is also affected by stage of development, environmental factors, storage of the fruits after harvest, fruit processing, cultivar, and the technique used for its measurement (Lee and Kader 2000; Alós *et al.* 2014; Giangriego *et al.* 2016). In orange peel the content of ascorbic acid increases during development, whereas the reverse occurs in the flesh (Alós *et al.* 2014). In apples the ascorbic acid content also varies between the different tissues of the fruit, and is higher in the peel (Li *et al.* 2009). In tomato fruits, changes in ascorbic plus DHA content during development appear to depend on the cultivar, and in some the content increases during ripening while in others it does not (Ioannidi *et al.* 2009; Mellidou *et al.* 2012). In peach, ascorbate decreases during development (Imai *et al.* 2009). In kiwifruit, total ascorbate concentration is dependent on both the species and stage of development (Bulley *et al.*, 2009).

B. Tartaric Acid

1. Occurrence and Functions of Tartaric Acid in Plants. Tartaric acid is present in plants from a wide range of families, and occurs in fruits, leaves, and other tissues (Stafford 1959, 1961; Loewus 1999; Mikulic-Petkovsek *et al.* 2012). However, its accumulation is sporadic, and it can be present in some cultivars of a species but be undetectable or at very low abundance in others (Stafford 1961; Saito and Loewus 1989;

Melgarejo *et al.* 2000; Mikulic-Petkovsek *et al.* 2012). In the leaves of nine species in which tartaric acid was present its concentration was 0.75–30 mg g^{-1} FW (Stafford 1959). Tartaric acid is particularly abundant in fruits of grape and tamarind (Ford 2012; Van den Bilcke *et al.* 2014). The metabolic functions of tartaric acid in fruits and other plant tissues are uncertain (DeBolt *et al.* 2007; Ford 2012). However, potentially its high content in some fruits could discourage both herbivory and attack by pathogens. In grape berries the bulk of the tartaric acid content appears to be located in the vacuole (Moskowitz and Hrzadina 1981; De Angeli *et al.* 2013; DeBolt *et al.* 2004; Fortes *et al.* 2011; Ford 2012). Further, in grape berries no evidence was found for the occurrence of crystalline forms of tartrate (DeBolt *et al.* 2004).

2. Synthesis of Tartaric Acid. Tartaric acid can be synthesized by at least two pathways in plants, and which pathway is utilized depends on the plant species (Loewus 1999; Ford 2012). A simplified scheme showing these pathways is shown in Fig. 8.7. Among fruits, tartaric acid metabolism has only been studied in detail in grape (Ruffner 1982a; Loewus 1999; Ford 2012). The bulk of the tartaric acid content in grape berries is synthesized within the fruit from ascorbic acid (Fig. 8.7), and this has been reviewed in detail (Loewus 1999; Ford 2012).

3. Occurrence and Contents of Tartaric Acid in Fruits. Tartaric acid is present in a wide range of fruits (Melgarejo *et al.* 2000; Koyuncu 2004; Nour *et al.* 2010; Gundogdu *et al.* 2011; Mikulic-Petkovsek *et al.*

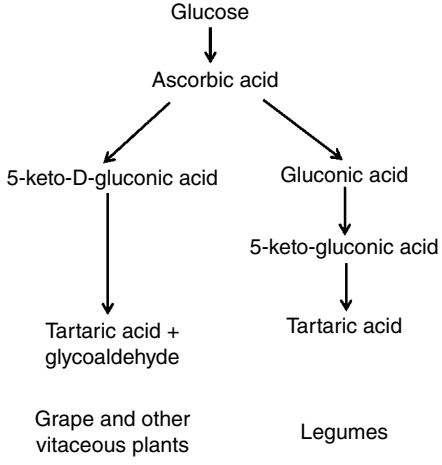

Fig. 8.7. Simplified scheme showing the synthesis of tartaric acid.

Table 8.5. Concentrations of tartaric acid (TA: mg·g^{-1} FW) in the flesh of some ripe fruits.

Fruits	TA level	Reference
Bilberry	1.85	Mikulic-Petkovsek *et al.* 2012
Blackberry	0	Mikulic-Petkovsek *et al.* 2012
Blueberry (highbush)	0	Mikulic-Petkovsek *et al.* 2012
Cherry (sweet)	0.08	Mahmood *et al.* 2012
Citrus (several types)	0.01–0.38	Nour *et al.* 2010
Cranberry (American)	1.97	Mikulic-Petkovsek *et al.* 2012
Currant (black)	1.44	Mikulic-Petkovsek *et al.* 2012
Currant (red)	0.2–0.5	Nour *et al.* 2011
Currant (red)	0.36	Mikulic-Petkovsek *et al.* 2012
Currant (white)	0.15	Mikulic-Petkovsek *et al.* 2012
Elderberry	0.44	Mikulic-Petkovsek *et al.* 2012
Goji berry	0	Mikulic-Petkovsek *et al.* 2012
Grape	5–22	Ruffner 1982; Wen *et al.* 2014
Gooseberry (white)	0.02	Mikulic-Petkovsek *et al.* 2012
Jostaberry	0.23	Mikulic-Petkovsek *et al.* 2012
Kiwifruit	0	Mikulic-Petkovsek *et al.* 2012
Ligonberry	3.34	Mikulic-Petkovsek *et al.* 2012
Mulberry (black)	0	Mikulic-Petkovsek *et al.* 2012
Mulberry (various)	0.10–1.18	Mahmood *et al.* 2012
Pomegranate	0.26–1.01	Aarabi *et al.* 2008
Plum (Japanese)	0.04	Singh *et al.* 2009
Raspberry	0.09	Mikulic-Petkovsek *et al.* 2012
Rowanberry	0.37	Mikulic-Petkovsek *et al.* 2012
Strawberry	0.5–1.8	Sturm *et al.* 2003
Strawberry	0.48–0.56	Mahmood *et al.* 2012
Strawberry	0–0.09	Mikulic-Petkovsek *et al.* 2012
Tamarind	84–124	El-Siddig 2006

2012; Ford 2012; Arena *et al.* 2013; Fawole and Opara 2013a,b). Some typical values for the tartaric acid concentration in the ripe flesh of a range of fruits are shown in Table 8.5. For those fruits studied it is clear that the content of tartaric acid is often dependent on the genotype/cultivar of a given type of fruit. Further, this content varies between tissues of a fruit and is also dependent on their stage of development (Kliewer *et al.* 1967; El-Siddig 2006; Van den Bilcke *et al.* 2014). In addition, tartaric acid content can also potentially be affected both by the conditions under which the fruits were grown and by storage and processing of the fruit after harvest. For several fruits some of these factors are considered below.

Grape. In the edible parts of ripe grapes the bulk of their organic acid content comprises malic and tartaric acids, and the concentration of tartaric acid is 5–22 mg g^{-1} FW (Ruffner 1982a; Melino *et al.* 2009; Wen

et al. 2014). However, the contents of these acids differ considerably among grape cultivars, and some can contain predominantly tartaric acid, others mainly malic acid whilst some contain large amounts of both (Kliewer *et al.* 1967). In grape pericarp the bulk of the tartrate content is synthesized before the onset of ripening (DeBolt *et al.* 2006; Melino *et al.* 2009). During ripening, the tartaric acid concentration (g^{-1} FW) decreases but usually this is largely a dilution effect arising from an increase in the size of the edible parts, and there is little change in the total tartrate content of the edible parts (Possner and Kliewer 1985; Iland and Coombe 1988; Rojas-Lara and Morrison 1989; Diakou *et al.* 1997; de Souza *et al.* 2005). Nevertheless, it is possible that there can be a decrease in total tartaric acid content of the edible parts at higher environmental temperatures (Ford 2012).

Other fruits. In the flesh of the Japanese plum ('Amber Jewel') the concentration of tartaric acid was 0.04 mg g^{-1} FW, and this did not decrease during ripening (Singh *et al.* 2009). In blueberry (*Vaccinium corymbosum*), the tartaric acid concentration was around 0.2 mg g^{-1} FW in white fruit, and this decreased during ripening (Forney *et al.* 2012). In a wide range of citrus fruits the tartaric acid concentration was 0.01–0.38 mg g^{-1} juice (extracted by squeezing half a fruit) (Nour *et al.* 2010). Tartaric acid is quite abundant (about 0.70 mg g^{-1} FW) in mature Ponkan mandarin (*Citrus reticulata*) flesh (Chen *et al.* 2012). The tartaric acid concentration of the edible parts of different pomegranate genotypes was reported to be 0–1.07 mg ml^{-1} juice (Melgarejo *et al.* 2000; Aarabi *et al.* 2008; Mena *et al.* 2011). In pomegranate 'Ruby,' the amount of tartaric acid was 2.9 mg ml^{-1} in the juice (minus seed) of the edible parts in immature fruits and this declined to 1.9 mg ml^{-1} in ripe fruit (Fawole and Opara 2013a,b). Tartaric acid is present in the flesh of a range of mulberry genotypes (Koyuncu 2004). The tartaric acid concentration in various mulberries was 0.1–1.18 mg g^{-1} FW (Mahmood *et al.* 2012). In *Berberis buxifolia* the tartaric acid concentration was about 1.5 mg g^{-1} FW in immature fruits, and about fivefold less in mature fruits (Arena 2013). However, a large part of this decrease is likely a dilution effect arising from the increase in the size of the fruit. Tartaric acid is very abundant in both tamarind fruits and leaves (Patnaik 1974; El-Siddig 2006; Van den Bilcke *et al.* 2014). In tamarind fruits the reported concentration range of tartaric acid is 31–124 mg g^{-1} FW, though the content varies between cultivars and does not decrease during development (El-Siddig 2006; Van den Bilcke *et al.* 2014).

C. Oxalic Acid

1. Functions of Oxalic Acid in Fruits and Other Plant Tissues. Oxalic acid has diverse unrelated functions in plants, and these functions are dependent on both the plant species and the tissue/cell type. Oxalic acid can function in calcium storage, protection from animals, tolerance to toxic concentrations of metal ions in the soil, pH regulation associated with nitrate assimilation, as a substrate for the production of hydrogen peroxide in the extracellular matrix (that is used both by peroxidases to crosslink polymers and in fungal defense responses), amongst others (Noonan and Savage 1999; Webb 1999; Ma *et al.* 2001; Franceschi and Nakata 2005; Tian *et al.* 2008; Proietti *et al.* 2009; Burbridge *et al.* 2014; Nakata 2015). In kiwifruit, the content of calcium oxalate crystals in the skin is higher than in the flesh, and these could serve as a deterrent to animal attack (Rassam and Laing 2005; Nguyễn and Savage 2013b). In grape berries the bulk of oxalic acid content is contained in crystals of calcium oxalate in idioblasts (DeBolt *et al.* 2004; Ford 2012), and these could potentially function as a store for calcium (DeBolt *et al.* 2004). Oxalate can be broken down by oxalate oxidase which produces CO_2 and peroxide, and this peroxide could be important in the oxidation of phenols in the extracellular matrix (Noonan and Savage 1999). This process could contribute to browning of the seed coat of grape seeds (DeBolt *et al.* 2004).

2. Synthesis and Catabolism of Oxalic Acid in Plants. Oxalic acid can potentially be synthesized by a number of metabolic pathways in plants (Franceschi and Horner 1980; Xu *et al.* 2006). It now appears that which pathway is utilized depends on tissue/cell type (Yu *et al.* 2010; Miyagi *et al.* 2013; Eprintsev *et al.* 2015). In plants, oxalate can be synthesized from either ascorbate or isocitrate (Yu *et al.* 2010; Miyagi *et al.* 2013; Eprintsev *et al.* 2015). Potentially, oxalate can also be synthesized either from oxaloacetate or from glyoxylate derived from photorespiration, though whether these pathways are utilized has been questioned (Franceschi and Loewus 1995; Yu *et al.* 2010). Oxalate is broken down by either oxalate oxidase, oxalate decarboxylase or oxalyl-CoA synthetase, and which is utilized appears to depend on both the plant species and tissue/cell type (Franceschi and Loewus 1995; Foster *et al.* 2016). Breakdown by oxalate decarboxylase appears to be a minor or insignificant pathway in plants (Franceschi and Loewus 1995). The most likely pathways utilized in oxalic synthesis and breakdown in plants are shown in Fig. 8.8.

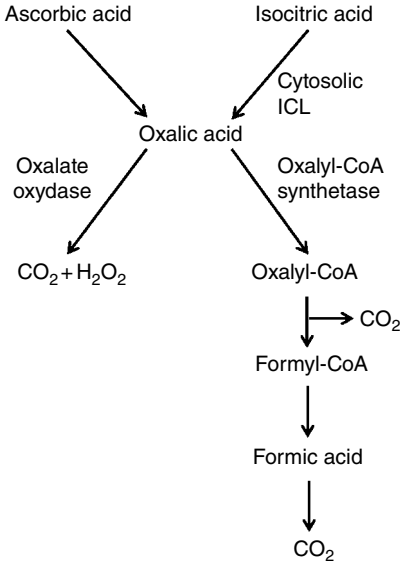

Fig. 8.8. Simplified scheme showing the synthesis and breakdown of oxalic acid. CoA = Coenzyme A: ICL = Isocitrate lyase.

In a large number of plant tissues, oxalic acid accumulates as crystals of calcium oxalate which are localized in the vacuole of certain cells called *idioblasts* (Franceschi and Horner 1980). In those idioblasts that have been studied, oxalic acid is synthesized from ascorbic acid. These crystals can potentially function as both a store of calcium, and also discourage herbivory (Loewus 1999; Kostman *et al.* 2001; DeBolt *et al.* 2004). In at least some idioblasts it appears that oxalate is broken down by the oxalyl-CoA synthetase pathway (Foster *et al.* 2016).

Large amounts of both soluble and insoluble oxalate are accumulated in the leaves of a number of plants such as dock, spinach, sorrel, rice, and rhubarb (Franceschi and Horner 1980; Tian *et al.* 2008; Rahman and Kawamura 2011). Many plants accumulate some oxalate in their leaves when grown using nitrate as opposed to ammonium as the nitrogen source (Clark 1936; Bennet-Clark 1937; Pucher and Vickery 1949). Recent studies have provided strong evidence that in the leaves of dock (*Rumex obtusifolius*) and some other plants oxalate is synthesized by a pathway that utilizes glyoxylate arising from the action of a cytosolic isocitrate lyase (Yu *et al.* 2010; Miyagi *et al.* 2013; Eprintsev *et al.* 2015). In these leaves it appears that a major function of oxalate is in pH regulation associated with the assimilation of nitrate (Franceschi

and Horner 1980; Tian *et al.* 2008; Rahman and Kawamura 2011). The assimilation of nitrate into amino acids consumes protons, and the protons produced by the synthesis of oxalic acid are thought to neutralize this alkalinity. In many plants malic acid is used instead of oxalic acid in this process (Franceschi and Horner 1980; Rahman and Kawamura 2011). The reason why some plants use oxalic rather than malic acid in this process is uncertain.

3. Occurrence and Contents of Oxalic Acid in Fruits. In plant tissues oxalic acid can be present either as the soluble form or as crystals of calcium oxalate, and the bulk of both these forms is located in the vacuole (Franceschi and Horner 1980; Wagner 1981; Matoh *et al.* 1987; Shen *et al.* 2002; Li *et al.* 2003). The measurement of the oxalate content of plant tissues can be problematic for two reasons. First, the correct procedure should be used to solubilize calcium oxalate crystals during extraction. Second, several techniques used to measure oxalate are unreliable, and it is difficult to say what proportion of oxalate is soluble or insoluble, as noted by De Bolt *et al.* (2004) and Nguyễn and Savage (2013a,b).

Oxalic acid is of widespread (and likely ubiquitous) occurrence in fruits, but in most of these it is not abundant. Some typical values for the oxalic acid concentrations in the ripe flesh of a range of fruits are shown in Table 8.6. In the edible parts of many commonly grown fruits the concentration of total oxalic acid (soluble + insoluble) is 0.02–0.6 mg g^{-1} FW (Noonan and Savage 1999; Nguyễn and Savage 2013a; D'Evoli *et al.* 2013, 2015; Abdel-Moemin 2014; Attalla *et al.* 2014; Cruz-Castillo *et al.* 2014). In other fruits the concentration of total oxalate can be moderate to very high: carambola (*Averrhoa carambola* L., star fruit; 4.36 mg g^{-1} FW), goji berries (*Lycium barbarum* L.; 1.38 mg g^{-1} FW) and Indian gooseberries (*Phyllanthus emblica* L.; 75.66 mg g^{-1} FW) (Nguyễn and Savage 2013a). Other horticultural crops that have high oxalic acid concentrations are spinach leaves (6 mg g^{-1} FW) and rhubarb petioles (6.4 mg g^{-1} FW) (Nguyễn and Savage 2013a; Attalla *et al.* 2014).

For those fruits in which it has been studied, it is clear that the content of oxalic acid is often dependent on the genotype/cultivar of a given type of fruit (Rassam *et al.* 2007; Guil-Guerrero and Rebolloso-Fuentes 2009). Further, this content varies between tissues of a fruit, and is also dependent on stage of development (Watanabe and Takahashi 1998; Nguyễn and Savage 2013a,b). In addition, oxalic acid content can also be affected both by the conditions under which the fruit was grown (De Kreij *et al.* 1992), and by the storage and processing of the fruit after harvest. For some fruits some of these factors are considered below.

Table 8.6. Concentrations of total oxalic acid ($mg \cdot g^{-1}$ FW) in the flesh of some ripe fruits (based on Nguyễn and Savage 2013a).

Fruits	OA level
Blueberry (highbush)	0.032
Boysenberry	0.105
Carambola	4.36
Currant (black)	0.109
Currant (red)	0.302
Cherry (sweet)	0.040–0.063
Feijoa	0.599
Goji berry	1.38
Gooseberry	0.281
Grape	0.029–0.039
Indian gooseberry	75.67
Kiwifruit (kiwi berry)	0.426
Kiwifruit (green)	0.200
Kiwifruit (gold)	0.153
Mangosteen	0.087
Persimmon	0.074
Pineapple	0.051
Plum (European)	0.114
Raspberry (red)	0.181
Tomato (red)	0.032
Rhubarb (petiole)	6.40
Tamarind (sweet)	0.062

The abundance of oxalic acid can vary between the different tissues of a given fruit. In kiwifruit the total oxalic acid contents are much higher in both the skin and seeds than in the flesh, and this difference is largely a result of the higher insoluble oxalate contents of the skin and seeds (Nguyễn and Savage 2013a,b). In kiwifruit, insoluble oxalate is largely present as crystals of calcium oxalate, and in the seeds these are concentrated in the outer cell layers such as the seed coat (Watanabe and Takahashi 1998; Rassam and Laing 2005; Nguyễn and Savage 2013b). Red gooseberry contains largely soluble oxalate, whereas in blueberry the situation is reversed (Nguyễn and Savage 2013a). In leaves, an increased supply of calcium to the plant increases the ratio of insoluble to soluble oxalate (Rahman and Kawamura 2011). Increasing the supply of calcium to tomato plants increases the fruit's content of calcium oxalate crystals (De Kreij *et al.* 1992). In peppers, a combination of high nitrogen supply and shading can lead to an increase in the abundance of calcium oxalate crystals in localized areas of the flesh (Aloni *et al.* 1994).

The oxalate content of different cultivars of a given fruit, such as kiwifruit, can vary (Rassam *et al.* 2007). Similarly, the oxalate content

of ripe tomatoes can differ between cultivars (Guil-Guerrero and Rebolloso-Fuentes 2009). The concentration of oxalic acid (g^{-1} FW) in kiwifruit decreases in different parts of the fruit during development (Watanabe and Takahashi 1998; Nguyễn and Savage 2013a). In banana, mume, persimmon and fig, the concentrations of oxalic acid (g^{-1} FW) decrease as the fruit matures (Wyman and Palmer 1964; Shimokawa *et al.* 1972; Marriot and Palmer 1980; Yamanaka *et al.* 1983; Watanabe and Takahashi 1998). In litchi (*Litchi chinensis*), oxalic acid was detected only in young fruits (Wang *et al.* 2006). In grape berries ('Shiraz') the content of oxalic acid per berry increased until the onset of ripening and then, depending on the season (or sampling procedure), either increased or decreased as the berries ripened (Melino *et al.* 2009).

In *Berberis buxifolia*, oxalate concentrations (g^{-1} DW) decreased during development (Arena 2013), but this decrease appeared to be a dilution effect that arises from an increase in the DW of the fruit. The oxalate concentration (g^{-1} FW) at several stages of development of the flesh + skin of four stone fruits (apricot, plumcot [Japanese plum-apricot hybrid], Japanese plum and peach) have been reported (Bae *et al.* 2014). In all of these it appears that the oxalate content of the entire flesh + skin increases for most of their development (Bae *et al.* 2014). In plumcot, at later stages of development, the oxalate content of the entire flesh + skin declined (Bae *et al.* 2014).

D. Galacturonic Acid

Galacturonic acid accounts for about 70% of the mass of pectins (Mohnen 2008), and pectin is a structurally complex family of polysaccharides that accounts for about one-third of materials present in the primary cell walls of dicots and non-poales monocots (Mohnen 2008). In ripe tomato pericarp the galacturonic acid content is around 30 mg per 100 mg cell wall (Gross and Wallner 1979). The content of pectin in ripe fruits has been considered in detail, and a typical value for many ripe fruits is about 6 mg g^{-1} FW (Baker 1997). However, there can be a considerable variation among cultivars of a given fruit, and some apple cultivars contain about 30 mg g^{-1} FW pectin (Rop *et al.* 2011). These values for pectin content would equate to galacturonic acid concentrations of 4.2–21.0 mg g^{-1} FW. Pectin is an important component of cell walls, and plays multiple roles in their function (Mohnen 2008). The presence of large amounts of galacturonic acid in cell walls is likely to contribute to determining the apoplastic pH and the buffering capacity of the apoplast. Pectin is synthesized in the Golgi lumen from nucleotide sugars, and these are attached to growing chains by glycosyltransferases.

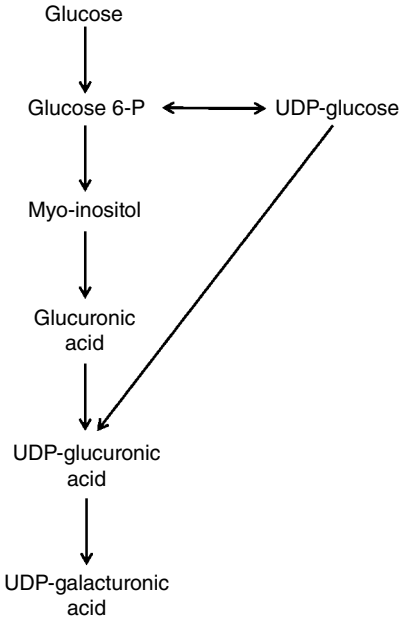

Fig. 8.9. Simplified scheme showing the synthesis of galacturonic acid. UDP = Uridine diphosphate.

Pectin is then transported to the cell wall in vesicles (Mohnen 2008). The nucleotide sugar utilized in the incorporation of galacturonic acid into pectin is UDP-galacturonate. Two pathways can be used in the synthesis of UDP-galacturonate: one pathway proceeds from the intermediate myo-inositol, and the second pathway via UDP-glucose (Fig. 8.9; after Loewus and Murthy 2000; Pieslinger *et al.* 2010).

V. FATTY ACIDS

Although most fruits do not accumulate large amounts of lipid in their flesh, some such as olive, avocado and oil palm do. In avocado flesh the oil content can typically be 20 mg g^{-1} FW (Ozdemir and Topuz 2004), while in oil palm mesocarp it can account for 85% of the dry weight (Dussert *et al.* 2013). Fatty acids are synthesized from acetyl-CoA in the plastid. Acetyl-CoA is converted to malonyl-CoA, which is added to the growing fatty acid chain; the addition of malonyl-CoA is then repeated until the chain is achieved of the desired length (Rawsthorne 2002).

VI. MALIC, CITRIC, AND METABOLICALLY RELATED ACIDS

A. Krebs Cycle Acids

For simplicity, this group will be referred to as the Krebs cycle acids because most of them can act as intermediates of the Krebs cycle (Nitsch 1953). This group of organic acids is of ubiquitous occurrence in fruits, and in most fruits one or two of these acids account for a large proportion of their organic acid content (Vickery and Pucher 1940; Thimann and Bonner 1950; Nitsch 1953; Ulrich 1971; Sweetman *et al.* 2009; Etienne *et al.* 2013; Famiani *et al.* 2015). The Krebs cycle acids that are accumulated in fruits are usually malic and/or citric acid. However, in some blackberry genotypes isocitric and lactoisocitric acids are the abundant acids (Curl and Nelson 1943; Fan-Chiang 1999), while in saskatoon (*Amelanchier alnifolia*) succinic is the most abundant organic acid (up to 16 mg g^{-1} FW; Rogiers and Knowles 1997). Malic and citric acids in fruits have been the subject of several recent reviews which also contain information on other aspects of this area (Fernie and Martinoia 2009; Sweetman *et al.* 2009; Etienne *et al.* 2013; Famiani *et al.* 2015).

1. Synthesis of Krebs Cycle Acids. The bulk of the Krebs cycle acids that are accumulated in fruits are thought to be synthesized from imported sugars within the fruit (Fig. 8.10) (Ruffner 1982b; Lobit *et al.* 2006; Sweetman *et al.* 2009; Ford 2012; Etienne *et al.* 2013; Famiani

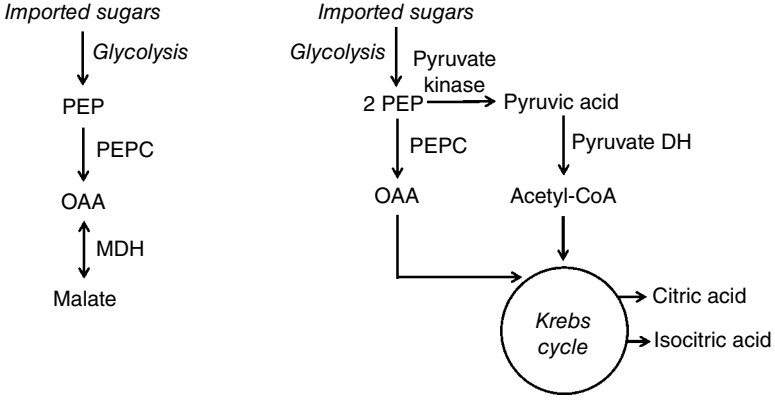

Fig. 8.10. Simplified scheme showing the synthesis of Krebs cycle acids. MDH = Malate dehydrogenase; OAA = Oxaloacetate, PEP = Phosphoenolpyruvate; CoA = Coenzyme A; PEPC = phosphoenolpyruvate carboxylase.

et al. 2015). Sugars enter glycolysis and are converted to phosphoenolpyruvate (PEP), which is then converted to oxaloacetate (OAA) in the cytosol by the enzyme phosphoenolpyruvate carboxylase (PEPC); OAA is then converted to malate by cytosolic malate dehydrogenase. If malate is being accumulated it is transported across the tonoplast into the vacuole, in which it is stored (Ruffner 1982b; Law and Plaxton 1995; Terrier and Romieu 2001; Sweetman *et al.* 2009; Etienne *et al.* 2013; Famiani *et al.* 2015). In contrast, if citrate is being accumulated, one molecule of PEP is converted to OAA by PEPC and a second PEP is converted to acetyl CoA. The latter and OAA are then combined by citrate synthase in the mitochondrion to produce citrate, which is subsequently transported across the tonoplast into the vacuole, in which it is stored (Etienne *et al.* 2013). Isocitrate is synthesized from citrate by aconitase, an enzyme which can be located in both mitochondria and cytosol (Gallardo *et al.* 1995; Sadka *et al.* 2000; Etienne *et al.* 2013).

2. Subcellular Location of Krebs Cycle Acids in Fruits. The vacuole can occupy 90–99% of the volume of parenchyma cells of ripening flesh of many fruits (Terrier and Romieu 2001; Sweetman *et al.* 2009; Etienne *et al.* 2013). The isolation of vacuoles from apples has shown that more than 90% of the malate is located in the vacuole (Yamaki 1984), and similarly malate is abundant in the vacuole of subepidermal cells of grape berries (Moskowitz and Hrzadina 1981). It is thought that the cytoplasmic concentration of malate in fruits is in the range 0.1–6.0 mM (Lobit *et al.* 2006). Studies on crassulacean acid metabolism (CAM) plants indicate that when malate is being synthesized by PEPC the cytoplasmic concentration of malate is in the range of 0.5–2.0 mM (Hafke *et al.* 2003). In grape berry, the K_i for inhibition of PEPC by malate is of this order (Diakou *et al.* 2000), and this is consistent with the concentration of malate in the cytoplasm of fruit cells being 0.1–6.0 mM when malate is being synthesized (Lobit *et al.* 2006). Thus, the consensus is that the bulk of the malate content in the flesh of fruits is located in the vacuole (Ruffner 1982b; Lobit *et al.* 2006; Sweetman *et al.* 2009; Etienne *et al.* 2013; Famiani *et al.* 2015). Transport processes at the tonoplast are an important factor in determining the vacuolar contents of these acids (Ruffner 1982b; Moing *et al.* 1999; Terrier and Romieu 2001; Lobit *et al.* 2006). Nevertheless, metabolism in the cytoplasm can also be important and the relative importance of these two factors in determining vacuolar content could differ between fruits (Etienne *et al.* 2013). For example, the abundance of mitochondrial and cytosolic aconitase could play a role in determining the citric acid content of citrus fruits (Sadka *et al.* 2000; Etienne *et al.* 2013), and studies

in tomato with altered amounts of phosphoenolpyruvate carboxykinase (PEPCK) have shown that changing the abundance of this enzyme alters the fruit's content of malic acid (Osorio *et al*. 2013; Huang *et al*. 2015a,b).

3. Breakdown and Utilization of Krebs Cycle Acids. The breakdown of stored citrate, including its conversion to malate, has recently been reviewed (Etienne *et al*. 2013). As far as malate is concerned, in fruits there are two pools of this compound: one pool is rapidly metabolized, whereas the other larger pool is not, and these correspond to the cytoplasmic and vacuolar pools (Farineau and Laval-Martin 1977). The enzymes that metabolize malate are located in the cytoplasm, and not the vacuole, and hence malate is only thought to be metabolized when it is released from the vacuole (Sweetman *et al*. 2009; Etienne *et al*. 2013; Famiani *et al*. 2015). The enzymes PEPCK and malic enzyme (ME) are utilized in the breakdown of malate in many fruits (Ruffner 1982b; Famiani *et al*. 2005, 2015; Sweetman *et al*. 2009; Etienne *et al*. 2013). In plants, PEPCK is only known to be located in the cytosol (Walker and Chen 2002; Leegood and Walker 2003). However, there are three forms of ME: a mitochondrial NAD-ME and both cytosolic and plastidic forms of NADP-ME (Famiani *et al*. 2005, 2012, 2015; Sweetman *et al*. 2009; Drincovich *et al*. 2010; Etienne *et al*. 2013). All three forms of ME are present in tomato flesh, in which their activities appear to be comparable (Jeffery *et al*. 1986; Knee and Finger 1992; Knee *et al*. 1996; Bahrami *et al*. 2001; Osorio *et al*. 2013). However, tomato flesh contains about 50-fold more NAD-ME activity than the flesh of either grape or peach (Borsani *et al*. 2009; Osorio *et al*. 2013; Biais *et al*. 2014; Sweetman *et al*. 2014).

In the flesh of fruits there are many uses for the PEP and pyruvate produced by either these enzymes or via glycolysis (Walker *et al*. 2015). Clearly, how they are utilized is dependent on the tissue; for example, in olive flesh the synthesis of lipid is a major process, whereas in the stone of peach the synthesis of lignin via the shikimate pathway is more prevalent. In the ripening flesh of grape, radiolabeling experiments indicate that a large proportion of this pyruvate and PEP are oxidized to CO_2 via the Krebs cycle (Ruffner 1982b; Famiani *et al*. 2016c). Similarly, for ripening tomato flesh, a comparison of the amount of glycolytic flux and the amount of CO_2 released from tomato fruits (Chalmers and Rowan 1971; Campbell *et al*. 1990), indicates that a large proportion of pyruvate produced by glycolysis is oxidized to CO_2. In the flesh of fruits, gluconeogenesis is thought to occur when malate/citrate/isocitrate are released from the vacuole and the amount of PEP produced from their metabolism exceeds the demands of other

processes (Ruffner 1982b; Walker *et al*. 2015). Hence, in grape pericarp a higher proportion of malate is utilized via gluconeogenesis at lower temperatures because the demands of the Krebs cycle are lower (Ruffner 1982b).

4. Metabolic Functions of Krebs Cycle Acids in Fruits. A large proportion of the Krebs cycle acid content of the flesh of fruits could have a non-metabolic function in altering the palatability of the flesh (see Section II. The function of the flesh of fruits and its implication for their organic acid contents). Nevertheless, this protective function must be superimposed on other underlying metabolic functions.

Contribution of Stored Krebs Cycle Acids to the Metabolic Requirements of the Flesh of Fruits. In grape pericarp the actual amount of malate dissimilated, together with the amount of CO_2 released by the fruit throughout ripening, were determined. This allowed the contribution of dissimilated malate to the amount of CO_2 released by the berry to be determined at different stages of ripening (Famiani *et al*. 2014a, 2016c). Stored malate could provide only a small proportion of the CO_2 released by the berry when the whole ripening period was considered, and sugars were required as a substrate for metabolism at all stages of ripening (Famiani *et al*. 2014a, 2016c). A similar approach was used for peach flesh, and this showed that stored malate and citrate could only provide a very small fraction of the substrate used by metabolism (Famiani *et al*. 2016b). It can be deduced, by comparing the amounts of CO_2 released from the fruit with the decrease in malate/citrate contents, that the situation is similar in tomato fruits (Chalmers and Rowan 1971; Campbell *et al*. 1990; Knee and Finger 1992; Biais *et al*. 2014). However, it is important to note that the quantitative importance of the contribution of organic acids to the substrate requirements of the flesh of fruits is dependent on the stage of development (Famiani *et al*. 2014a, 2016a). In grape, stored malate can provide a relatively large proportion of this substrate for a short period of time soon after veraison, after which its contribution is lower for the rest of ripening (Famiani *et al*. 2014a, 2016a). Moreover, in grape, there is evidence indicating that the actual contribution of malate is dependent on the cultivar, and this contribution is higher in cultivars which contain more malate at the start of ripening and have a shorter ripening period (Famiani *et al*. 2016c).

Contribution of Krebs Cycle Acids to the Provision of CO_2 for Photosynthesis. PEPCK and the malic enzymes are utilized as decarboxylases in the photosynthetic CO_2-concentrating mechanisms of

both C_4 and CAM plants (Edwards *et al.* 1971; Dittrich *et al.* 1973; Leegood and Walker 1999, 2003). The presence of these, and other enzymes utilized in photosynthesis in C_4 and CAM plants, has led to the suggestion that they could function similarly in fruits (for references, see Blanke and Lenz 1989). However, the presence of these enzymes in fruits, together with certain aspects of malate metabolism, has been attributed to a role in the regulation of energy utilization, pH regulation, aspartate synthesis and, to a limited extent, internal CO_2 concentration rather than a role in photosynthesis, also considering that only the skin would receive enough light to carry out photosynthesis (Blanke and Lenz 1989).

Gluconeogenesis and the Contribution of Stored Krebs Cycle Acids to Sugar Accumulation. Gluconeogenesis from Krebs cycle acids has been shown to occur in the flesh of several fruits (Farineau and Laval-Martin 1977; Ruffner 1982b; Leegood and Walker 1999; Leegood *et al.* 1999; Osorio *et al.* 2013; Huang *et al.* 2015a,b). The first steps in gluconeogenesis from malate involve its conversion to PEP, which in plants requires either MDH and PEPCK or ME together with PPDK (Walker and Chen 2002; Leegood and Walker 2003). In both cases, the PEP so produced is converted to sugars by a reversal of the reactions of glycolysis (Plaxton 1996). In the flesh of grape, apricot, peach, plum, cherry and soft fruits, PEPCK is present, whereas PPDK appears to be either not present or at very low abundance (Famiani *et al.* 2005, 2009, 2014b, 2016c; Famiani and Walker 2009; Baldicchi *et al.* 2015). Both, PEPCK and PPDK polypeptides were present in tomato (Famiani *et al.* 2016a). These results suggest that in those fruits studied (except tomato) the bulk of any gluconeogenic flux proceeds via PEPCK, whereas in tomato both PEPCK and PPDK could potentially be utilized. PPDK was also detected in the fruit of cactus pear (*Opuntia ficus-indica*) (Walker *et al.* 2011c); however, this plant is a CAM plant and PPDK functions in its photosynthesis.

If the amount of malate and citrate, that arose from the decrease in their contents during the ripening of the flesh of some fruits such as gooseberry, were converted to sugars by gluconeogenesis, then this would give rise to up to 20% of the sugars accumulated in the ripe fruit (Famiani *et al.* 2009). However, in reality this contribution of gluconeogenesis to sugar accumulation will be much lower. This is because a large proportion of the dissimilated malate and citrate is not used by gluconeogenesis but is used by catabolic processes such as the Krebs cycle. Further, in many fruits there is little or no decrease in their malate and citrate contents during ripening (Ruffner *et al.* 1975; Halinska and

Frenkel 1991; Famiani *et al.* 2016b,c). Hence, in grape, peach, and tomato the contribution of stored malate/citrate to the net accumulation of sugars during ripening is very small (Ruffner 1982b; Famiani *et al.* 2016b,c). In grape, this contribution is likely to be less than 1% (Famiani *et al.* 2016c). However, this appears to be at variance with the finding that changing the abundance of PEPCK in tomato fruits has a quite large effect on their organic acid and/or sugar contents (Osorio *et al.* 2013; Huang *et al.* 2015a,b). This apparent contradiction can be resolved if altering the abundance of PEPCK also alters processes other than gluconeogenesis. During the ripening of fruits there is evidence that there are times when malate is synthesized and transported into the vacuole, and other times when malate is released from the vacuole and is utilized (Famiani *et al.* 2005; Walker *et al.* 2015). This process could be linked to nitrogen metabolism (Famiani *et al.* 2016b). Hence, altering the abundance of PEPCK could affect these processes. In keeping with this view a range of metabolic fluxes are also altered in tomato flesh when the abundance of PEPCK is reduced (Osorio *et al.* 2013). Similarly, soluble sugar contents were markedly altered in ripe tomato fruits that contained low amounts of glutamate dehydrogenase (Ferraro *et al.* 2015). Therefore, how changing the abundance of PEPCK in tomato flesh alters sugar and organic acid contents is likely to be complex.

For many years glycolysis was thought to be inhibited in ripening grape, and that malate provided the bulk of the substrate used by metabolism. Gluconeogenesis was thought to occur because there was an excess of malate (Ruffner 1982b; Sweetman *et al.* 2009). However, recent studies have shown this to be untrue and that sugars provide the bulk of the substrate used by metabolism (Famiani *et al.* 2014a, 2016c). One explanation for the occurrence of gluconeogenesis in fruits is that malate flux from the vacuole is not constant during ripening, and there are times when there is an enhanced efflux of malate which results in an excess of malate in the cytosol (Famiani *et al.* 2015, 2016b; Walker et al. 2015).

5. The Role of Krebs Cycle Acids in Nitrogen Metabolism. The Krebs cycle acids and their close relatives are intimately associated with nitrogen metabolism in plants (Chibnall 1939; Vickery and Pucher 1940). Thus, oxaloacetate, 2-oxoglutarate and pyruvate form the basis of the carbon skeletons of many amino acids and amides (Lea 1993). Amino acids can be divided into several groups on the basis of their synthesis, and many amino acids are derived from aspartate, glutamate, and pyruvate. These three metabolites are readily interconverted by the reversible enzymes, aspartate aminotransferase, alanine aminotransferase and malate dehydrogenase (Lea 1993).

The Krebs cycle acids are also associated with nitrogen metabolism because they are utilized in pH regulation necessary for certain aspects of nitrogen metabolism (Raven and Smith 1976; Gerendás and Ratcliffe 2000, 2013; Stitt *et al*. 2002). Thus, in the leaves of many C_3 plants the content of Krebs cycle acids is often largely dependent on whether the plant is grown on nitrate or ammonium (Clark 1936; Bennet-Clark 1937; Pucher and Vickery 1949; Kandlbinder *et al*. 1997). The increased malate content in the leaves of plants grown on nitrate is a consequence of pH regulation associated with the assimilation of nitrate (Bennet-Clark 1937; Raven and Smith 1976; Kandlbinder *et al*. 1997; Stitt *et al*. 2002). The assimilation of nitrate into most amino acids consumes one proton, whereas the assimilation of ammonium produces one proton (Raven and Smith 1976). In the source leaves of plants that are grown on nitrate and assimilate it in the leaf, nitrate enters the leaf via the xylem. The assimilation of nitrate into amino acids consumes protons, and this is counteracted by the synthesis of malic acid in the leaf. The malate is then exported to the root (leaving protons in the leaf). In addition, the export of glutamate and aspartate from the leaf, rather than their amides glutamine and asparagine, also contributes to counteracting the consumption of protons in the leaf. This is because more protons are produced in the synthesis of glutamate and aspartate compared to glutamine and asparagine, and glutamate and aspartate are often converted to their amides in vascular tissues before delivery to developing sink tissues (Walker and Chen, 2002; Chen *et al*. 2004). The metabolism of malate in the root consumes protons, and this is counteracted by the excretion of alkali into the soil (Raven and Smith 1976). If plants are grown on ammonium this can be assimilated in either the root, stem, or the leaf, and this depends on various factors such as the plant species (Schjoerring *et al*. 2002). Ammonium is transported to the leaf via the xylem, along with malate that is synthesized in the root. The utilization of this malate in the leaf then counteracts acidity arising from the assimilation of ammonium into amino acids (Raven and Smith 1976).

Potentially, the Krebs cycle acid content of fruits could be altered by the growth of the plant on either nitrate or ammonium. For most of their development, tomato fruits from plants grown using nitrate as their nitrogen source have higher contents of both malate and citrate, than those of plants supplied with only ammonium as their nitrogen source (Caraňgal *et al*. 1954; Xu *et al*. 2012). This is consistent with malic and citric acids being synthesized in the fruits and being utilized to counteract the proton-consuming effect of growth on nitrate. It is worth considering how much malic/citric acid would be required to counteract the amount of protons consumed in the hypothetical case of

all organic forms of nitrogen in the fruit being synthesized from nitrate in the fruit. In reality, a large amount of the nitrogen imported into most fruits is in the form of glutamine and asparagine (Roubelakis-Angelakis and Kliewer 1992; Famiani *et al.* 2012). The flesh of many fruits typically contains about 4 mg g^{-1} FW organic nitrogen compounds (i.e., proteins, amino acids, and amides). The molecular masses of amino acids, malate and citrate are roughly of the same order. The synthesis of one molecule of amino acid consumes on average one proton, whereas the synthesis of malic acid produces two protons and citric acid three protons. Thus, as a rough approximation, less than 2 mg g^{-1} FW malic + citric acid are required to counteract the alkalinity resulting from the synthesis of all the organic nitrogenous compounds from nitrate. The contents of these acids in the flesh of fruits is often much greater than this, and thus it is feasible for pH perturbations arising from the assimilation of nitrate to be counteracted in this way. Bollard (1971) noted that there are deviations between titratable acidity and the contents of organic acids in the flesh of many fruits, and the import of different forms of nitrogenous material could contribute to these.

6. Occurrence and Contents of Krebs Cycle Acids in Fruits. The Krebs cycle acids are of ubiquitous occurrence in fruits (Vickery and Pucher 1940; Thimann and Bonner 1950; Nitsch 1953; Ulrich 1971; Sweetman *et al.* 2009; Etienne *et al.* 2013; Famiani *et al.* 2015). Some typical values for the malic and citric acid concentrations in the ripe flesh of a range of fruits and leaves are shown in Tables 8.7 and 8.8. For those fruits in which it has been studied, it is clear that the concentrations of these acids are often dependent on the genotype/cultivar of a given type of fruit. Further, these contents vary between the tissues of a fruit, and are also dependent on their stage of development (Tables 8.9 and 8.10). The Krebs cycle acid content of the flesh of fruits can also be affected by other conditions under which the fruit was grown, and also by storage and processing of the fruit after harvest (Sweetman *et al.* 2009; Etienne *et al.* 2013; Famiani *et al.* 2015). For several fruits some of these factors are considered below.

Stone fruits (apricot, cherry, peach and plum). The bulk of the organic acid content of the edible parts of most stone fruits consists of malic and/or citric acids. However, appreciable amounts of quinic acid are present in some stone fruits (De Moura and Dostal 1965; Van Gorsel *et al.* 1992; Moing *et al.* 1998; Gurrieri *et al.* 2001; Walker *et al.* 2011a; Famiani *et al.* 2012). In peach and apricot, both malate and citrate are present in appreciable amounts (Moing *et al.* 1998; Baldicchi *et al.*

Table 8.7. Concentrations of malic acid (MA; mg g^{-1} FW) in the flesh of some ripe temperate fruits and some leaves and roots. For leaves and roots, nitrate and ammonium refer to the form of nitrogen on which the plants were grown.

Fruits	MA level	Reference
Apple	4.8–14.7	Fuleki *et al.* 1994
Blackberry	0.5–6.0	Wrolstad *et al.* 1980
Blueberry (highbush)	0.47–0.50	Markakis *et al.* 1963
Blueberry (lowbush)	5.0	Kalt and McDonald 1996
Cherry (sweet)	5.0–8.5	Girard and Kopp 1998
Cherry (sweet)	8.5–10	Kelebek and Selli 2011
Cherry (sweet)	6.3–14	Ballistreri *et al.* 2013
Citrus (orange)	0.6–2.0	Ting and Attaway 1971
Citrus (tangerine)	1.8–2.1	Ting and Attaway 1971
Citrus (grapefruit)	0.4–0.6	Ting and Attaway 1971
Citrus (lemon)	1.7–2.6	Ting and Attaway 1971
Citrus (lime)	2.0	Ting and Attaway 1971
Currant (black)	0.7–1.7	Rodriguez *et al.* 1992
Currant (red)	0.5–2.1	Rodriguez *et al.* 1992
Currant (red)	0.3–0.5	Nour *et al.* 2011
Currant (white)	0.4	Rodriguez *et al.* 1992
Peach	8.0	Byrne *et al.* 1991
Peach	2.0–7.0	Dirlewanger *et al.* 1999
Pear	1.6–2.0	Drake and Eisele 1999
Plum (damson)	7.0	García-Mariño *et al.* 2008
Plum (European)	20.0	Lombardi-Boccia *et al.* 2004
Plum (Japanese)	7.6–15.9	Robertson *et al.* 1992
Plum (Japanese)	8.0–10.0	Singh *et al.* 2009
Raspberry	0.14–1.7	Rodriguez *et al.* 1992
Strawberry	1.5–2.8	Moing *et al.* 2001
Leaves		
Tobacco (nitrate)	11.0	Pucher and Vickery 1949
Tobacco (ammonium)	0.8	Pucher and Vickery 1949
Barley (nitrate)	4.3	Kandlbinder *et al.* 1997
Barley (ammonium)	0.3	Kandlbinder *et al.* 1997
Roots		
Barley (nitrate)	<0.13	Kandlbinder *et al.* 1997
Barley (ammonium)	<0.13	Kandlbinder *et al.* 1997

2015; Famiani *et al.* 2016b). By contrast, cherries and plums contain mainly malate (Walker *et al.* 2011a; Famiani *et al.* 2012). In all stone fruits, the concentration (amount in g^{-1} FW) of total organic acids in the flesh increases up to ripening and then decreases (Moing *et al.* 1998, 1999; Garcia Mariño *et al.* 2008; Singh *et al.* 2009; Walker *et al.* 2011a; Famiani *et al.* 2012). In cherry flesh, the reduction in concentration of malate during ripening is only due to a dilution effect brought about by expansion of the parenchyma cells. The total amount per fruit increases, showing that there is a net synthesis (Walker *et al.* 2011a). In both plum

Table 8.8. Concentrations of citric acid (CA; mg g^{-1} FW) in the flesh of some ripe temperate fruits and some leaves. For leaves, nitrate and ammonium refer to the form of nitrogen on which the plants were grown.

Fruits	CA level	Reference
Apple	0.3–0.5	Fuleki *et al.* 1994
Blueberry (highbush)	4.8–5.8	Markakis *et al.* 1963
Blueberry (lowbush)	5.0	Kalt and McDonald 1996
Citrus (orange)	5.6–9.8	Ting and Attaway 1971
Citrus (tangerine)	8.6–12.2	Ting and Attaway 1971
Citrus (grapefruit)	11.9–21.0	Ting and Attaway 1971
Citrus (lemon)	40.0–43.8	Ting and Attaway 1971
Citrus (lime)	0.8	Ting and Attaway 1971
Currant (black)	26.0–38.0	Rodriguez *et al.* 1992
Currant (red)	20.0–28.0	Rodriguez *et al.* 1992
Currant (red)	25.0–31.0	Nour *et al.* 2011
Currant (white)	20.0	Rodriguez *et al.* 1992
Cherry (sweet)	0.02–0.07	Girard and Kopp 1998
Cherry (sweet)	1.4–2.7	Kelebek and Selli 2011
Peach	3.0	Byrne *et al.* 1991
Peach	0.4–3.0	Dirlewanger *et al.* 1999
Pear	3.0–3.6	Drake and Eisele 1999
Plum (damson)	0.05	García-Mariño *et al.* 2008
Plum (European)	0.25–0.27	Lombardi-Boccia *et al.* 2004
Plum (Japanese)	0.20	Robertson *et al.* 1992
Plum (Japanese)	0.12	Singh *et al.* 2009
Raspberry	14.0–20.0	Rodriguez *et al.* 1992
Strawberry	4.4–10.5	Sturm *et al.* 2003
Strawberry	6.0–10.0	Moing *et al.* 2001
Leaves		
Tobacco (nitrate)	1.5	Pucher and Vickery 1949
Tobacco (ammonium)	0.2	Pucher and Vickery 1949

Table 8.9. Concentrations of malic acid (MA; mg g^{-1} FW) in the flesh of unripe and ripe fruits. For unripe fruit (except tomato) the maximum content during the period of development studied is given. For tomato, unripe fruit are 28 days after anthesis, and nitrate and ammonium denote the form of nitrogen with which the plant was fertilized.

Fruits	Unripe	Ripe	Reference
Apricot	32.0	8.0	Baldicchi *et al.* 2015
Blueberry (highbush)	3.1	0.6	Famiani *et al.* 2005
Cherry (sweet)	8.0	6.7	Walker *et al.* 2011a
Currant (red)	5.5	1.9	Famiani *et al.* 2005
Gooseberry	11.0	4.3	Famiani *et al.* 2009
Grape	16.0–24.0	1.3–4.0	Famiani *et al.* 2000, 2014b
Peach	6.6	4.8	Famiani *et al.* 2016
Plum (Japanese)	17.0	11.0	Famiani *et al.* 2012
Raspberry	6.7	1.3	Famiani *et al.* 2005
Strawberry	2.0	2.6	Famiani *et al.* 2005
Tomato (nitrate)	19.0	5.0	Xu *et al.* 2012
Tomato (ammonium)	6.5	1.0	Xu *et al.* 2012

Table 8.10. Concentrations of citric acid (CA; mg g^{-1} FW) in the flesh of unripe and ripe fruits. For unripe fruit the maximum content during the period of development studied is given. For tomato, unripe fruit are 28 days after anthesis, and nitrate and ammonium denote the form of nitrogen with which the plant was fertilized.

Fruits	Unripe	Ripe	Reference
Apricot	16	10	Baldicchi *et al.* 2015
Blueberry (highbush)	24	13	Famiani *et al.* 2005
Currant (red)	26	21	Famiani *et al.* 2005
Gooseberry	13	9	Famiani *et al.* 2009
Peach	4.8	0.57	Famiani *et al.* 2016
Plum (Japanese)	0.12	0.06	Famiani *et al.* 2012
Raspberry	21	14	Famiani *et al.* 2005
Strawberry	7.7	7.3	Famiani *et al.* 2005
Tomato (nitrate)	10	10	Xu *et al.* 2012
Tomato (ammonium)	5.5	5.5	Xu *et al.* 2012

(malate) and apricot flesh (citrate and malate), the decrease in the concentration of organic acids during the first part of ripening arises from an increase in their size, and actually there is a net synthesis. A net dissimilation only occurs during the last part of ripening (Famiani *et al.* 2012; Baldicchi *et al.* 2015). In some peaches during ripening a net dissimilation of citrate occurs, whereas there is a net synthesis of malate (Famiani *et al.* 2016b).

Pome fruits (apple, medlar, pear, quince and other minor pome fruits). In apple, malic acid is the most abundant Krebs cycle acid, although smaller amounts of citric acid are present (Hulme and Wooltorton 1957; Ulrich 1971; Hecke *et al.* 2006; Petkovsek *et al.* 2007; Wu *et al.* 2007). In ripe apple flesh, the content of malic acid differs greatly among cultivars (Hecke *et al.* 2006; Petkovsek *et al.* 2007; Wu *et al.* 2007). The concentration (g^{-1} FW) of malic acid increases up to ripening and then decreases throughout ripening (Berüter 2004; Zhang *et al.* 2010). By contrast, the content per fruit increases for most of the development and only shows a slight decline just before harvest. This pattern was observed in both the cultivars 'Honeycrisp' (Zhang *et al.* 2010) and 'Golden Delicious' (F. Famiani *et al.*, unpublished data). The content of malic acid changes during storage of the fruit after harvest, though these changes are dependent on storage conditions. After storage under normal air for 3–6 months the malic acid concentration is lower than at harvest (Ackermann *et al.* 1992; Róth *et al.* 2007). However, this decrease can be reduced if the atmosphere surrounding the apples is modified (Róth *et al.* 2007).

In pears, either malic acid or citric acid can be the most abundant organic acid, and this depends on both the species and cultivar. In most

pears malic acid is the most abundant Krebs cycle acid, and in all pears it is present in relatively high amounts. By contrast, citric acid has a low abundance in some pears (Arfaioli and Bosetto 1993; Hudina and Štampar 2000; Chen *et al.* 2007; Lu *et al.* 2011; Sha *et al.* 2011a). A comparison of cultivars deriving from different pear species showed that on average the total organic acid concentration was highest in those derived from *Pyrus ussuriensis* (5.98 mg g^{-1} FW), followed by *Pyrus bretschneideri* (3.07 mg g^{-1} FW), *Pyrus pyrifolia* (2.66 mg g^{-1} FW), and *Pyrus communis* (2.42 mg g^{-1} FW) (Sha *et al.* 2011a). It seems that early-maturing European cultivars (derived from *Pyrus communis*) have a lower malic:citric acid ratio than late-maturing ones (Hudina and Štampar 2000). In general, the concentrations (g^{-1} FW) of both malate and citrate decrease during ripening, or at least a part of it (Arfaioli and Bosetto 1993; Sha *et al.* 2011b). However, the citric acid concentration (g^{-1} FW) of fruits of *Pyrus pyrifolia* (Lu *et al.* 2011) and both the citric and malic acid concentrations (g^{-1} FW) of fruits of *Pyrus bretschneideri* (Sha *et al.* 2011c) increased during ripening. During storage of Kuerle fragrant pear (*Pyrus serotina*) and Yali pear (*Pyrus bertschneideri*), the content of malic acid (the most abundant acid) increased during the first 3 months of storage and then decreased (Chen *et al.* 2006a,b).

In mature quince (*Cydonia oblonga* Mill.) flesh, malic acid is the predominant Krebs cycle acid, and the citric acid content is very low (Rodríguez-Guisado *et al.* 2009). Quince fruit also contains appreciable amounts of quinic acid (Szychowski *et al.* 2014). Recently, it was reported that phytic acid is the main organic acid in the quince juice of Spanish genotypes (Szychowski *et al.* 2014).

The organic acid content of other pome fruits, whose commercial cultivation is not as widespread, such as medlar (*Mespilus germanica* L.), service tree (*Sorbus domestica* L.), and rowanberry (*Sorbus aucuparia* L.), has been determined. In most of these fruits malic acid is usually the most abundant Krebs cycle acid at harvest (Barbieri *et al.* 2011). However, in medlars the citric acid content can be lower (Barbieri *et al.* 2011; Selcuk and Erkan 2015a,b) or higher than malic acid content (Glew *et al.* 2003a). During the development of medlars on the tree, citric acid concentration (g^{-1} FW) increased until ripening and then decreased. By contrast, malic acid concentration increased throughout development on the tree (Glew *et al.* 2003a). Medlar fruits are hard when harvested, and need to be stored for several weeks. This allows them to both soften and sweeten, and during this storage Krebs cycle acid concentration decreases (Glew *et al.* 2003b; Selcuk and Erkan 2015a,b).

Soft fruits. Citric, together with smaller amounts of malic acid, account for the bulk of the Krebs cycle acid content of blueberries (*Vaccinium corymbosum* L.), cranberries (*Vaccinium macrocarpon* Ait.), gooseberries (*Ribes grossularia* L.), raspberries (*Rubus ideaus* L.), white currants, redcurrants (*Ribes rubrum* L.), blackcurrants (*Ribes nigrum* L.), and strawberries (*Fragaria vesca* L.) (Whiting 1958; Green 1971; Famiani *et al.* 2005, 2009; Çelik *et al.* 2008; Nour *et al.* 2011; Mikulic-Petkovsek *et al.* 2012; Mazur *et al.* 2014). In blackberries, isocitric, lactoisocitric, citric and malic acids make up the bulk of their organic acid content; however, the abundance of each of these is dependent on the cultivar or species (Whiting 1958; Wrolstad *et al.* 1980; Fan-Chiang 1999; Kafkas *et al.* 2006).

During the ripening of blueberries, gooseberries, raspberries and redcurrants, the concentrations of both malic and citric acids (g^{-1} FW) decreased, whereas there was little change in these concentrations in strawberries and an increase in cranberries (Moing *et al.* 2001; Famiani *et al.* 2005, 2009; Çelik *et al.* 2008). The citric acid content per fruit increased throughout the development of strawberries and redcurrants, whereas for blueberries and raspberries it increased up to the first part of ripening and then decreased (Famiani *et al.* 2005). In blackcurrants, a steady increase in the concentration of citric acid (g^{-1} FW) occurred throughout development and ripening (Toldam-Andersen and Hansen 1997). Other less widely grown soft fruits are Japanese wineberry (*Rubus phoenicolasius* Maxim), jostaberry (*Ribes nidigrolaria*), and goji berry (wolfberry; *Lycium barbarum* L.), and in these citric acid was more abundant than malic acid (Mikulic-Petkovsek *et al.* 2012).

Grape. In ripe berries, malic and tartaric acids account for the bulk of the organic acid content of the flesh and skin (Ruffner 1982a,b). Malic acid is accumulated before ripening and there is a large decrease in its concentration (g^{-1} FW) during ripening (Ruffner 1982b; Famiani *et al.* 2014a,b; Rienth *et al.* 2016).

Kiwifruit. In ripening fruits of *Actinidia deliciosa* (which includes 'Hayward,' the most cultivated genotype), *A. chinensis*, *A. rufa* and *A. arguta*, citric acid is generally more abundant than malic acid (Okuse and Ryugo 1981; Reid *et al.* 1982; Walton and De Jong 1990; Ferrandino and Guidoni 1998; Marsh *et al.* 2009; Nishiyama *et al.* 2008). The concentration of malic acid (g^{-1} FW) is generally highest at mid-summer and then decreases towards harvest. Citric acid concentration (g^{-1} FW) also declines as harvest approaches (Okuse and Ryugo 1981; Walton and De Jong 1990; Marsh *et al.* 2009).

Tropical and subtropical fruits. In mango (*Mangifera indica* L.), citric and malic acids are the most abundant Krebs cycle acids during development. The loss of acidity at maturity is caused by a large reduction in citric acid content, and a small reduction in malic acid content (Medlicott and Thompson 1985). In kaki (*Dyospyros kaki* L.), the content of malic and/or citric acid increases until the beginning of ripening and then decreases (Daood *et al.* 1992). The most abundant organic acid in ripe bananas (*Musa* spp.) is malic (Morvai and Molnár-Perl 1992). Malic acid content is high at the consumption stage in mamey sapote (*Pouteria sapota* [(Jacquin) (H. E Moore & Steam]) (Arenas-Ocampo *et al.* 2007), and in loquat (*Eriobotrya japonica* Lindl) (Chen *et al.* 2009). In pineapple (*Ananas comosus* L.), citric acid is the most abundant organic acid at maturity, followed by malic acid (Bartolomé *et al.* 1995). Ripe papaya (*Carica papaya* L.) contains both citric and malic acids (González-Aguilar *et al.* 2003). In litchi (*Litchi chinensis* Sonn.) tartaric and malic acids are the most abundant organic acids at the time the fruit are consumed (Hu *et al.* 2005). Cherimola (*Annnona cherimola* Mill.) fruit after storage and at the time of consumption contains 6 mg g^{-1} FW malic acid and 3 mg g^{-1} FW citric acid (Alique and Zamorano 2000). In sugar apple *(Annona squamosa* L.), citric acid is abundant at the time of harvest and consumption (Bolivar-Fernández *et al.* 2009). In feijoa (*Feijoa sellowiana* Berg) fruit, both malic and citric acid are abundant at the time of consumption. In guava (*Psidium guajava* L.), citric acid content is high in both immature and mature fruits (Soares *et al.* 2007).

VII. CONCLUSIONS

All fruits contain a large number of different organic acids. These organic acids can be arranged into groups according to the pathways utilized in their synthesis. For a given fruit a small number of organic acids can be particularly abundant, and which of these organic acids are abundant can vary greatly between fruits of different species and among different cultivars. Further, this abundance is also dependent on the stage of development of the fruit and the part of the fruit in question. Fruits serve to protect the seed(s) and assist in their dispersal, and hence the accumulation of some organic acids in fruits could be related to these functions. This chapter provides an overview of the contents, metabolism, and functions of these organic acids in the flesh of fruits.

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